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PTO-1590 (9-90)

FILE 'REGISTRY' ENTERED AT 09:55:58 ON 20 MAR 2002
E ".BETA.-GALACTOSIDASE"/CN 5
L1 22 S ".BETA.-GALACTOSIDASE" ?/CN
E LISTERIOLYSIN/CN 5
L2 2 S E3-E4
E ACTA/CN 5
E ACT-A/CN 5
L3 24 S L1 OR L2

-key terms

FILE 'CAPLUS' ENTERED AT 09:57:13 ON 20 MAR 2002
L1 22 SEA FILE=REGISTRY ABB=ON PLU=ON ".BETA.-GALACTOSIDASE" ?/CN
L2 2 SEA FILE=REGISTRY ABB=ON PLU=ON (LISTERIOLYSIN/CN OR "LISTERIOLYSIN (LISTERIA MONOCYTOGENES CLONE PLI3 GENE LISA)"/CN)
L3 24 SEA FILE=REGISTRY ABB=ON PLU=ON L1 OR L2
L4 33013 SEA FILE=CAPLUS ABB=ON PLU=ON L3 OR (BETA OR B)(W)(GAL OR GALACTOSIDASE) OR LISTERIOLYSIN OR LISTERIO LYSIN OR HLY OR ACTA OR ACT A OR LACZ OR LAC Z
L5 416 SEA FILE=CAPLUS ABB=ON PLU=ON L4 AND ((SALMONELL? OR S)(W)TYPHI? OR TY21A OR SL7207 OR LT2 OR 33275 OR TY 21A OR SL 7207 OR LT 2)
L6 44 SEA FILE=CAPLUS ABB=ON PLU=ON L5 AND VECTOR
L7 35 SEA FILE=CAPLUS ABB=ON PLU=ON L6 AND (CMV? OR PCMV? OR PLASMID)

L7 ANSWER 1 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:282122 CAPLUS
DOCUMENT NUMBER: 135:327997
TITLE: Construction and characterization of Streptococcus suis-Escherichia coli shuttle cloning **vectors**
AUTHOR(S): Takamatsu, Daisuke; Osaki, Makoto; Sekizaki, Tsutomu
CORPORATE SOURCE: Laboratory of Molecular Bacteriology, National Institute of Animal Health, Tsukuba, 305-0856, Japan
SOURCE: Plasmid (2001), 45(2), 101-113
CODEN: PLSMDX; ISSN: 0147-619X
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB PSSU1, a native **plasmid** of Streptococcus suis DAT1, was used to construct pSET-series shuttle **vectors**. In addn. to the replication function of pSSU1, these **vectors** contain the multiple cloning sites and **lacZ'** gene from pUC19, which means that X-gal screening can be used to select recombinants in Escherichia coli. PSET1, pSET2, and pSET3 carry cat, spc, and both of these genes, resp., as selectable markers. These **vectors** could be introduced into S. suis, E. coli, **Salmonella typhimurium**, S. pneumoniae, and S. equi ssp. equi by electro-transformation. The recA gene was cloned from S. suis and sequenced, and this information was used in the construction of a recA mutant of S. suis. Transformation frequencies and/or **plasmid** stability of all pSET **vectors** tested were decreased in both S. suis and E. coli recA mutants compared with the parental strains. These results suggested that functional RecA protein improved the maintenance of

pSET **vectors** in both S. suis and E. coli. Moreover, cloning of the functional S. suis recA gene into pSET2 and complementation anal. of the recA mutant were successful in S. suis but not in E. coli. These results showed that pSET **vectors** are useful tools for cloning and analyzing S. suis genes in S. suis strains directly. (c) 2001 Academic Press.

IT **369406-20-2P**
RL: BPN (Biosynthetic preparation); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); PREP (Preparation); USES (Uses)
(amino acid sequence; of proteins encoded by genes located on Streptococcus suis-Escherichia coli shuttle cloning **vectors** (pSET1, pSET2, and pSET3))

REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 2 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:495292 CAPLUS
DOCUMENT NUMBER: 133:234950
TITLE: Constitutively expressed phoP inhibits mouse-virulence of **Salmonella typhimurium** in an Spv-dependent manner
AUTHOR(S): Matsui, Hidenori; Kawakami, Takatoshi; Ishikawa, Satoshi; Danbara, Hirofumi; Gulig, Paul A.
CORPORATE SOURCE: Laboratory of Infectious Diseases and Immunology, Center for Basic Research, The Kitasato Institute, Tokyo, 108-8642, Japan
SOURCE: Microbiol. Immunol. (2000), 44(6), 447-454
CODEN: MIIMDV; ISSN: 0385-5600
PUBLISHER: Center for Academic Publications Japan
DOCUMENT TYPE: Journal
LANGUAGE: English

AB In **Salmonella typhimurium**, the transcription of several virulence genes including spvB is regulated by the PhoP/PhoQ regulatory system. To further examine the relationship between the PhoP/PhoQ and Spv systems for virulence in mice, we examd. a non-polar phoP mutation combined with different virulence **plasmid** genotypes for effects on virulence of **S. typhimurium** in the mouse model. PhoP-/Spv+ and PhoP-/Spv- mutants were not detectably recovered from the spleens of s.c. or orally inoculated mice. The phoP gene constitutively expressed from the **lacZ** promoter of a low copy no. **vector** (phoPc) only partially complemented the non-polar phoP mutation for mouse-virulence in both the Spv+ and Spv- backgrounds; both PhoPc strains exhibited virulence equal only to a PhoP+/Spv- strain. Interestingly, in a PhoP+ background, the phoPc gene reduced splenic infection of the Spv+ but not Spv- salmonellae after s.c. or oral inoculation compared with the PhoP+ parents. Addnl., the phoPc gene in an Spv+ background reduced the net growth of salmonellae in macrophages in vitro; phoPc in an Spv- background was without effect. These data suggest that the constitutive expression of the phoP gene attenuates the virulence of **S. typhimurium** in mice in an Spv-dependent manner.

REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 3 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:402012 CAPLUS
DOCUMENT NUMBER: 133:54547
TITLE: Bacterial oxygen-responsive transcription regulators for control of gene expression in bacteria and mammalian cells
INVENTOR(S): Green, Jeff; Guest, John; Barker, Mike; Lewis, Claire; Sumner, Stephanie
PATENT ASSIGNEE(S): Oxford Biomedica (UK) Limited, UK
SOURCE: PCT Int. Appl., 57 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000034488	A1	20000615	WO 1999-GB4068	19991206

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: GB 1998-26775 A 19981204
AB A nucleic acid **vector** is provided comprising at least one therapeutic gene operably linked to a bacterial transcription regulator DNA-binding site and, optionally, one or more nucleotide sequences of interest encoding a bacterial transcription regulator for binding to said binding site. The bacterial transcriptional regulator is responsive to oxygen and/or redox levels, such as the gene fnr and flp systems. FNR up-regulates the expression of a reporter gene in response to anoxia in a strain of **Salmonella typhimurium**. Replacement of the reporter **lacZ** gene with a therapeutic gene will enable prokaryotes such as attenuated strains of bacteria transformed with constructs of the invention toe used as whole cell **vectors** for delivery of therapeutic gene products to animal or human patients. 293 Cells were also transfected with mammalian expression **plasmid** pCIneo-fnr, which encodes FNR, and exhibits hypoxia-regulated regulation of gene expression.
REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 4 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:45769 CAPLUS
DOCUMENT NUMBER: 132:221037
TITLE: Cloning, expression, and immune responses of a hybrid antigen corresponding to hepatitis C virus and hepatitis B virus
AUTHOR(S): Huang, Jiansheng; Xu, Zhun; Saleh, Said A.; Xie, Yongmei; Zhang, Minghui; Ren, Daming
CORPORATE SOURCE: National Laboratory of Genetic Engineering,

Institute of Genetics, Fudan University, Shanghai, 200433, Peop. Rep. China
SOURCE: Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao (1999), 15(6), 881-884
CODEN: ZSHXF2; ISSN: 1007-7626
PUBLISHER: Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao Bianweihui
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
AB In order to explore. the possibility of HCV/HBV. bivalent vaccine, a synthetic multi-epitopes antigen gene PCX of HCV was fused with HBsAg gene. The recombinant gene PCXS was cloned into a . **beta.-galactosidase** expression **vector** pWR450-1 to highly express a fusion protein GZ-PCXS in E. coli JM105 and attenuated **Salmonella typhimurium** SL3261, whose products were specifically recognized by anti-HBs and anti-HCV antibodies. After GZ-PCXS antigen and recombinant SL3261 (pWR/PCXS) bacteria were used to immunize ICR mice, high level of anti-GZ-PCXS IgG was induced by GZ-PCXS antigen. Oral live SL3261(pWR/PCXS) bacteria also elicited significant proliferation responses of CD8+ T cells. All the immunized mice were safe without obvious toxicity. The results showed that the possibility of the bivalent of HCV/HBV vaccines and oral live vaccine might be the best route for this purpose.

L7 ANSWER 5 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:651471 CAPLUS
DOCUMENT NUMBER: 132:1959
TITLE: Use of in vivo-regulated promoters to deliver antigens from attenuated Salmonella enterica var. Typhimurium
AUTHOR(S): Dunstan, Sarah J.; Simmons, Cameron P.; Strugnell, Richard A.
CORPORATE SOURCE: Department of Microbiology and Immunology, The University of Melbourne, Parkville, 3052, Australia
SOURCE: Infect. Immun. (1999), 67(10), 5133-5141
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB This study describes the construction and anal. of three in vivo-inducible promoter expression **plasmids**, contg. pnirB, ppagC, and pkatG, for the delivery of foreign antigens in the .DELTA.aroAD mutant of Salmonella enterica var. Typhimurium (hereafter referred to as **S. typhimurium**). The reporter genes encoding **.beta.-galactosidase** and firefly luciferase were used to assess the comparative levels of promoter activity in **S. typhimurium** in vitro in response to different induction stimuli and in vivo in immunized mice. It was detd. that the ppagC construct directed the expression of more **.beta.-galactosidase** and luciferase in **S. typhimurium** than the pnirB and pkatG constructs, both in vitro and in vivo. The gene encoding the C fragment of tetanus toxin was expressed in the aroAD mutant of **S. typhimurium** (BRD509) under the control of the three promoters. Mice orally immunized with attenuated **S. typhimurium** expressing C fragment under control of the pagC

promoter [BRD509(pKK/ppagC/C frag)] mounted the highest tetanus toxoid-specific serum antibody response. Levels of luciferase expression in vivo and C-fragment expression in vitro from the pagC promoter appeared to be equiv. to if not lower than the levels of expression detected with the constitutive trc promoter. However, mice immunized with BRD509(pKK/ppagC/C frag) induced significantly higher levels of tetanus toxoid-specific antibody than BRD509(pKK/C frag)-immunized mice, suggesting that the specific location of foreign antigen expression may be important for immunogenicity. Mutagenesis of the ribosome binding sites (RBS) in the three promoter/C fragment expression **plasmids** was also performed. Despite optimization of the RBS in the three different promoter elements, the expression levels in vivo and overall immunogenicity of C fragment when delivered to mice by attenuated **S. typhimurium** were not affected. These studies suggest that in vivo-inducible promoters may give rise to enhanced immunogenicity and increase the efficacy of **S. typhimurium** as a vaccine **vector**.

REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 6 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:386054 CAPLUS
DOCUMENT NUMBER: 131:154690
TITLE: Role of human N-acetyltransferases, NAT1 or NAT2, in genotoxicity of nitroarenes and aromatic amines in **Salmonella typhimurium** NM6001 and NM6002
AUTHOR(S): Oda, Yoshimitsu; Yamazaki, Hiroshi; Shimada, Tsutomu
CORPORATE SOURCE: Osaka Prefectural Institute of Public Health, Osaka, 537-0025, Japan
SOURCE: Carcinogenesis (1999), 20(6), 1079-1083
CODEN: CRNGDP; ISSN: 0143-3334
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Human NAT1 and NAT2 genes were subcloned into pACYC184 **vector** and the **plasmids** thus obtained were introduced into **Salmonella typhimurium** O-acetyltransferase-deficient strain NM6000 (TA1538/1,8-DNP/pSK-1002), establishing new strains NM6001 and NM6002, resp. We compared the sensitivities of these two strains with those of NM6000 towards carcinogenic nitroarenes and arom. amines in the SOS/umu response. The induction of umuC gene expression by these chems. in the presence and absence of the S9 fraction was assayed by measuring the cellular **.beta.-galactosidase** activity expressed by the umuC"**lacZ** fusion gene in the tester strains. 2-Nitrofluorene and 2-aminofluorene induced umuC gene expression more strongly in the NM6001 strain than in the NM6002 strain. In contrast, induction of umuC gene expression by 1,8-dinitropyrene, 6-aminochrysene and 2-amino-3,5-dimethylimidazo[4,5-f]quinoline was weaker in the NM6001 strain than in the NM6002 strain. 1-Nitropyrene, 2-amino-6-methyl-dipyrido[1,2-a:3',2'-d]imidazole, 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole, 3-amino-1-methyl-5H-pyrido[4,3-b]indole, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine and 2-amino-3-methyl-9H-pyrido[2,3-

b]indole were found to induce umuC gene expression at similar extents in both strains. These results suggest that the newly developed strains can be employed for the studies on mechanisms of genotoxicity of a variety of nitroarenes and arom. amines, along with the assessment of cancer risk to humans.

REFERENCE COUNT: 44 THERE ARE 44 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 7 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:214242 CAPLUS
DOCUMENT NUMBER: 131:72361
TITLE: Introduction of protein or DNA delivered via recombinant **Salmonella typhimurium** into the major histocompatibility complex class I presentation pathway of macrophages
AUTHOR(S): Catic, Andre; Dietrich, Guido; Gentschev, Ivo; Goebel, Werner; Kaufmann, Stefan H. E.; Hess, Jurgen
CORPORATE SOURCE: Department of Immunology, University Clinics Ulm, Ulm, D-89070, Germany
SOURCE: Microbes Infect. (1999), 1(2), 113-121
CODEN: MCINFS; ISSN: 1286-4579
PUBLISHER: Editions Scientifiques et Medicales Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Recombinant (r) **Salmonella typhimurium** aroA strains which display the hen egg ovalbumin OVA257-264 peptide SIINFEKL in secreted form were constructed. In addn., attenuated rS. typhimurium pcDNA-OVA constructs harboring a eukaryotic expression **plasmid** encoding complete OVA were used to introduce the immunodominant OVA257-264 epitope into the major histocompatibility complex (MHC) class I presentation pathway. Both modes of antigen delivery (DNA and protein) by Salmonella vaccine carriers stimulated OVA257-264-specific CD8 T-cell hybridomas. An in vitro infection system was established that allowed both rSalmonella carrier devices to facilitate MHC class I delivery of OVA257-264 by coexpression of **listeriolysin** (**Hly**) or by coinfection with rS. typhimurium Hlys. Coexpression of **Hly** and coinfection with rS. typhimurium Hlys slightly improved MHC class I processing of OVA. Our data provide further evidence for the feasibility of attenuated, **Hly**-expressing rS. typhimurium carriers secreting heterologous antigens or harboring heterologous DNA as effective vaccines for stimulating CD8 T cells in addn. to CD4 T cells.
REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 8 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:744971 CAPLUS
DOCUMENT NUMBER: 130:13200
TITLE: Use of a secretion **vector** for fertility control by oral vaccination
INVENTOR(S): Donner, Peter; Goebel, Werner; Demuth, Andreas; Gentschev, Ivaylo; Hess, Juergen; Kaufmann, Stefan

PATENT ASSIGNEE(S): Schering A.-G., Germany
SOURCE: PCT Int. Appl., 33 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9850067	A2	19981112	WO 1998-EP2679	19980507
WO 9850067	A3	20000629		
W: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
DE 19720761	A1	19981112	DE 1997-19720761	19970507
AU 9879107	A1	19981127	AU 1998-79107	19980507
BR 9809244	A	20000627	BR 1998-9244	19980507
EP 1015023	A1	20000705	EP 1998-929286	19980507
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2002501507	T2	20020115	JP 1998-547740	19980507
NO 9905429	A	20000107	NO 1999-5429	19991105

PRIORITY APPLN. INFO.: DE 1997-19720761 A 19970507
WO 1998-EP2679 W 19980507

AB An oral antifertility vaccine uses attenuated Salmonellae or other gram-neg. attenuated vaccination strains which contain a secretion **vector** encoding fertility-inhibiting proteins or protein fragments. The **vector** preferably contains genes or gene fragments coding for zona pellucida proteins fused to the C-terminal transport signal (HlyAS) of the hemolysin (HlyA) of Escherichia coli, along with the complete hemolysin operon including the **hly**-specific promoter and an enhancer-like regulator hlyR. Expression of these proteins allows secretion of the fertility-inhibiting proteins across the inner and outer bacterial membranes into the extracellular medium. Different systems of expression are used which enable a specific MHCII/CD4 or MHCI/CD8 immune response to be generated. Thus, cDNA encoding human zona pellucida protein B (ZPB) was excised from **plasmid** pGEX-KG-huZPB and inserted into expression **vector** pMOhly1; the product was used to transform E. coli. Supernatant proteins from overnight cultures of the transformed E. coli were tested with polyclonal antibodies for the presence of hemolysin/ZP fusion proteins. Pos. pMOhly1 derivs. were used to transform **S. typhimurium** LB5000 lacking a restriction system, to allow efficient introduction of E. coli DNA. Recombinant expression **vectors** from **S. typhimurium** LB5000 were isolated and used to transform attenuated vaccination strain **S. typhimurium SL7207**, which subsequently expressed and secreted the hemolysin/ZP fusion protein.

L7 ANSWER 9 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:714930 CAPLUS
DOCUMENT NUMBER: 130:80092
TITLE: Gene transfer in dendritic cells, induced by

oral DNA vaccination with **Salmonella typhimurium**, results in protective immunity against a murine fibrosarcoma

AUTHOR(S): Paglia, Paola; Medina, Eva; Arioli, Ivano; Guzman, Carlos A.; Colombo, Mario P.

CORPORATE SOURCE: Division of Experimental Oncology D, Istituto Nazionale per Lo Studio e la Cura dei Tumori, Milan, I-20133, Italy

SOURCE: Blood (1998), 92(9), 3172-3176
CODEN: BLOOAW; ISSN: 0006-4971

PUBLISHER: W. B. Saunders Co.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A live attenuated AroA- auxotrophic mutant of **Salmonella typhimurium** (**SL7207**) has been used as carrier for the **pCMV**.beta. **vector** that contains the . **beta**.-**galactosidase** (.**beta**.-**gal**) gene under the control of the immediate early promoter of Cytomegalovirus (**CMV**). We tested whether orally administered bacterial carrier could enter and deliver the transgene to antigen-presenting cells (APCs) through the natural enteric route of infection and whether .**beta**.-**gal** expression could generate a protective response against an aggressive murine fibrosarcoma transduced with the .**beta**.-**gal** gene (F1.A11) that behaves operationally as a tumor-assocd. antigen. After three courses, at 15-day intervals, mice developed both cell-mediated and systemic humoral responses to .**beta**.-**gal**. Mice vaccinated with the Salmonella harboring **pCMV**.beta., but not with **plasmid**-less carrier, showed resistance to a challenge with F1.A11 cells. These expts. suggest that Salmonella-based DNA immunization allows us to specifically target antigen expression in vivo to APCs. To prove that the transgene is actually expressed by APCs as a function of an eukaryotic promoter, the green fluorescent protein (GFP) was placed under the control of either the eukaryotic **CMV** or a prokaryotic promoter. Using cytofluorometric anal., GFP was detected only in splenocytes of mice receiving a Salmonella carrier harboring GFP under the **CMV** promoter. These results indicate that transgene expression occurs because of a Salmonella-mediated gene transfer to eukaryotic cells. Finally, approx. 19% of the splenocytes expressed GFP. Among them, F4/80+ macrophages and CD11cbright dendritic cells (DCs) were scored as pos. for GFP expression. Extensive work has been performed trying to optimize the way to transfect DCs, ex vivo, with genes coding for relevant antigens. We show here, for the first time, that DCs can be directly and specifically transduced in vivo such to induce DNA vaccination against tumors.

REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 10 OF 35 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1998:709195 CAPLUS
DOCUMENT NUMBER: 129:329695

TITLE: An attenuated strain of **Salmonella typhimurium** carrying eukaryotic expression constructs for use in oral vaccines

INVENTOR(S): Darji, Ayub; Guzman, Carlos; Timmis, Kenneth;

Wehland, Jurgen; Weiss, Siegfried; Gerstel, Birgit; Chakraborty, Trinad; Wachholz, Petra
PATENT ASSIGNEE(S): Gesellschaft Fur Biotechnologische Forschung m.b.H., Germany
SOURCE: PCT Int. Appl., 52 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9848026	A1	19981029	WO 1997-EP6933	19971211
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9857562	A1	19981113	AU 1998-57562	19971211
EP 977874	A1	20000209	EP 1997-953786	19971211
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRIORITY APPLN. INFO.: EP 1997-106503 19970418
WO 1997-EP6933 19971211

AB An attenuated strain of **Salmonella typhimurium** has been developed for use as a vehicle for oral genetic immunization. Eukaryotic expression **vectors** contg. the genes for **.beta.-galactosidase**, or truncated forms of **ActA** and **listeriolysin** - two virulence factors of Listeria monocytogenes - that were controlled by an eukaryotic promoter have been used to transform a **S. typhimurium** aroA strain. Multiple or even single immunizations with these transformants induced a strong cytotoxic and helper T cell response as well as an excellent antibody response. Multiple immunizations with **listeriolysin** transformants protected the mice completely against a lethal challenge of L. monocytogenes. Partial protection was already obsd. with a single dose. **ActA** appeared not to be a protective antigen. The strength and the kinetics of the response suggested that the heterologous antigens were expressed within the eukaryotic host cells following transfer of **plasmid** DNA from the bacterial carrier strain. Transfer of **plasmid** DNA could be unequivocally shown in vitro using primary peritoneal macrophages. The demonstration of RNA splice products and expression of **.beta.-galactosidase** in the presence of tetracycline - an inhibitor of bacterial protein synthesis - indicated that the gene was expressed by host cells rather than bacteria. Oral genetic immunization with Salmonella carriers provides a highly versatile system for antigen delivery, represents a potent system to identify candidate protective antigens for vaccination, and will permit efficacious generation of antibodies against virtually any DNA segment encoding an open reading frame.

L7 ANSWER 11 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:709194 CAPLUS
DOCUMENT NUMBER: 129:326951
TITLE: Regulatory system for inducible expression of genes with lambdoid promoters
INVENTOR(S): Mertens, Nico Maurice August Corneel; Remaut, Erik Rene; Fiers, Walter Charles
PATENT ASSIGNEE(S): Vlaams Interuniversitair Instituut Voor Biotechnologie, Belg.
SOURCE: PCT Int. Appl., 39 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9848025	A2	19981029	WO 1998-EP2465	19980423
WO 9848025	A3	19990121		
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9876491	A1	19981113	AU 1998-76491	19980423
AU 729712	B2	20010208		
EP 975775	A2	20000202	EP 1998-924212	19980423
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2001526533	T2	20011218	JP 1998-545048	19980423

PRIORITY APPLN. INFO.: NL 1997-1005884 A 19970423
WO 1998-EP2465 W 19980423

AB The invention relates to a regulation system for inducible expression of genes, comprising a lambdoid promoter, a gene coding for a repressor for the lambdoid promotor and a gene coding for an antirepressor of the repressor, which antirepressor is under the influence of an inducible promotor. The invention further relates to a regulatory replicon, comprising said gene coding for an antirepressor, an expression system, comprising said regulatory replicon, and an expression **vector** based on a lambdoid promotor, and also to a method for producing a gene product in a heterologous host, by providing a culture of a host comprising a heterologous sequence which codes for the gene product, wherein the expression of the heterologous sequence is under the control of a regulation system, a gene coding for a repressor for the lambdoid promotor and a gene coding for an antirepressor, and by inducing the promotor of the antirepressor gene. Thus, a replicon (designated pICA2) is constructed comprising the gene coding for the **Salmonella typhimurium** phage P22 ant protein under the control of the PN25/O2 promoter, the lacIq gene under the control of the pLacIq promoter, and the gene coding for the cI857 repressor. The system is illustrated on the basis of the prokaryote **lacZ** gene and the eukaryote genes coding for human

interferon-.gamma., murine interleukin 2, and human interleukin 2 as model systems for protein synthesis.

L7 ANSWER 12 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:153110 CAPLUS
DOCUMENT NUMBER: 128:293790
TITLE: A **Salmonella typhimurium** strain genetically engineered to secrete effectively a bioactive human interleukin (hIL)-6 via the Escherichia coli hemolysin secretion apparatus
AUTHOR(S): Hahn, Heinz P.; Hess, Claudia; Gabelsberger, Josef; Domdey, Horst; von Specht, Bernd-Ulrich
CORPORATE SOURCE: Chirurgische Forschung, Chirurgische Universitatsklinik, Freiburg i. B., Germany
SOURCE: FEMS Immunol. Med. Microbiol. (1998), 20(2), 111-119
PUBLISHER: CODEN: FIMIEV; ISSN: 0928-8244
DOCUMENT TYPE: Elsevier Science B.V.
LANGUAGE: Journal
English

AB Human interleukin-6 (hIL-6) cDNA was genetically fused with the Escherichia coli hemolysin secretory signal (hlyAS) sequence in a **plasmid vector**. Recombinant E. coli XL-1 Blue and attenuated **Salmonella typhimurium** secreted a 30 kDa hIL-6-HlyAS fusion protein, with an addnl. form of higher apparent mol. mass produced by **S. typhimurium**. In **S. typhimurium** cultures hIL-6-HlyAS concns. entered a plateau at 500 to 600 ng mL-1 culture supernatant. In contrast to E. coli XL-1 Blue, in **S. typhimurium** culture supernatants hIL-6-HlyAS was accumulated faster reaching three-fold higher maximal concns. The cell proliferating activity of hIL-6-HlyAS fusion protein(s) was equiv. to that of mature recombinant hIL-6. Furthermore, hIL-6-secreting **S. typhimurium** were less invasive than the attenuated control strain. Therefore, the bulky hemolysin secretory peptide at the C-terminus of the fusion protein does not markedly affect hIL-6 activity, suggesting that the hemolysin secretion app. provides an excellent system to study immunomodulatory effects of in situ synthesized IL-6 in Salmonella vaccine strains.

L7 ANSWER 13 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:74060 CAPLUS
DOCUMENT NUMBER: 128:111790
TITLE: Metabolic Activation of Aromatic Amine Mutagens by Simultaneous Expression of Human Cytochrome P450 1A2, NADPH-Cytochrome P450 Reductase, and N-Acetyltransferase in Escherichia Coli
AUTHOR(S): Josephy, P. David; Evans, David H.; Parikh, Asit; Guengerich, F. Peter
CORPORATE SOURCE: Guelph-Waterloo Centre for Graduate Work in Chemistry, Departments of Chemistry and Biochemistry and of Molecular Biology and Genetics, University of Guelph, ON, N1G 2W1, Can.
SOURCE: Chem. Res. Toxicol. (1998), 11(1), 70-74
PUBLISHER: CODEN: CRTOEC; ISSN: 0893-228X
American Chemical Society

DOCUMENT TYPE: Journal
LANGUAGE: English
AB The authors describe the construction of a new strain of Escherichia coli designed to bioactivate arom. amines and to detect their mutagenicity with high sensitivity. Strain DJ4309 bears two **plasmids**, a pACYC184-derived **plasmid** which expresses **Salmonella typhimurium** acetyl CoA:arylamine N-acetyltransferase (NAT) and a pBR322-derived **plasmid** which expresses human cytochrome P 450 1A2 and NADPH-cytochrome P 450 reductase. The combined actions of these enzymes convert arom. amines into reactive, mutagenic N-acetoxy esters. The strain also carries a mutated copy of the **lacZ** gene (on an F' factor) which reverts to the wild-type gene by a -(GpC) frameshift mutation. Strain DJ4309 expresses high levels of NAT and cytochrome P 450 1A2 and is very sensitive to mutagenesis induced by representative arom. amines. Mutagenicity of 2-aminoanthracene in strain DJ4309 is higher than what can be obtained by rat liver homogenate 9000g supernatant (S9) activation in the parent strain lacking the P 450 expression **vector**. Strain DJ4309 provides a useful system for detecting mutagenic arom. amines and for studying their metab. by human P 450 1A2.

L7 ANSWER 14 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:809173 CAPLUS
DOCUMENT NUMBER: 128:113825
TITLE: Oral somatic transgene vaccination using attenuated **S. typhimurium**
AUTHOR(S): Darji, Ayub; Guzman, Carlos A.; Gerstel, Birgit; Wachholz, Petra; Timmis, Kenneth N.; Wehland, Jurgen; Chakraborty, Trinad; Weiss, Siegfried
CORPORATE SOURCE: Division of Cell Biology and Immunology, Gesellschaft fur Biotechnologische Forschung, National Research Centre for Biotechnology, Braunschweig, D-38124, Germany
SOURCE: Cell (Cambridge, Mass.) (1997), 91(6), 765-775
CODEN: CELLB5; ISSN: 0092-8674
PUBLISHER: Cell Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB An attenuated strain of **S. typhimurium** has been used as a vehicle for oral genetic immunization. Eukaryotic expression **vectors** contg. truncated genes of **ActA** and **listeriolysin**-two virulence factors of Listeria monocytogenes-have been used to transform **S. typhimurium** aroA. Multiple or even single oral immunizations with such transformants induced excellent cellular and humoral responses. In addn., protective immunity was induced with **listeriolysin** transformants. The quality of the responses suggested a transfer of **plasmid** DNA from the bacterial carrier to the host. Such transfer was unequivocally shown in vitro with primary peritoneal macrophages. We describe a highly versatile system for antigen delivery, identification of protective antigens for vaccination, and efficient generation of antibodies against the product of open reading frames present on virtually any DNA segment.

L7 ANSWER 15 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:721137 CAPLUS
DOCUMENT NUMBER: 126:27415

TITLE: Development of antigen-delivery systems, based on the Escherichia coli hemolysin secretion pathway
AUTHOR(S): Gentschev, Ivaylo; Mollenkopf, Hans; Sokolovic, Zeljka; Hess, Juergen; Kaufmann, Stefan H. E.; Goebel, Werner
CORPORATE SOURCE: Lehrstuhl fuer Mikrobiologie, Theodor-Boveri-Institut fuer Biowissenschaften, Am Hubland, D-97074, Wurzburg, Germany
SOURCE: Gene (1996), 179(1), 133-140
CODEN: GENED6; ISSN: 0378-1119
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB We describe the development of **plasmid vectors** carrying the expression sites, an hlyA cassette and the secretion genes of Escherichia coli hemolysin. These allow the synthesis and secretion of heterologous microbial antigens in E. coli and attenuated Salmonella aroA strains. Genes or gene fragments encoding microbial antigens are inserted in-frame into a residual part of the hlyA gene which essentially encodes the HlyA secretion signal (HlyAs). In general, the fused genes, carrying the hlyAs sequence at the 3' terminus, are efficiently expressed, and the synthesized antigens are secreted into the culture supernatant of the producing strain. Attenuated Salmonella strains synthesizing either HlyAs-fused **listeriolysin** or p60 of Listeria monocytogenes were constructed by this procedure and shown to provide protective immunity against L. monocytogenes in mice. The most effective protection was obtained when these microbial antigens were secreted by the attenuated Salmonella strains. We further present new approaches which may allow the application of this antigen-delivery system to any microbial antigen.

L7 ANSWER 16 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:114492 CAPLUS
DOCUMENT NUMBER: 124:168026
TITLE: A new **Salmonella typhimurium** NM5004 strain expressing rat glutathione S-transferase 5-5: use in detection of genotoxicity of dihaloalkanes using an SOS/umu test system
AUTHOR(S): Oda, Yoshimitsu; Yamazaki, Hiroshi; Thier, Ricarda; Ketterer, Brian; Guengerich, F. Peter; Shimada, Tsutomu
CORPORATE SOURCE: Osaka Prefectural Inst. Public Health, Osaka, 537, Japan
SOURCE: Carcinogenesis (1996), 17(2), 297-302
CODEN: CRNGDP; ISSN: 0143-3334
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The Escherichia coli mu operon was subcloned into a pKK233-2 **vector** contg. rat glutathione S-transferase (GST) 5-5 cDNA and the **plasmid** thus obtained was introduced into **Salmonella typhimurium** TA1535. The newly developed strain **S. typhimurium** NM5004, was found to have 52-fold greater GST activity than the original umu strain **S. typhimurium** TA1535/pSK1002. We compared sensitivities of these two tester strains, NM5004 and

TA1535/pSK1002, for induction of umuC gene expression with several dihaloalkanes which are activated or inactivated by GST 505 activity. The induction of umuC gene expression by these chems. was monitored by measuring the cellular .**beta**.-**galactosidase** activity produced by umuC''**lacZ** fusion gene in these two tester strains. Ethylene dibromide, 1-bromo-2-chloroethane, 1,2-dichloroethane, and methylene dichloride induced umuC gene expression more strongly in the NM5004 strain than the original strain. 4-Nitroquinoline 1-oxide and N-methyl-N'-nitro-N-nitrosoguanidine were found to induce umuC gene expression to similar extents in both strains. In the case of 1-nitropyrene and 2-nitrofluorene, however, NM5004 strain showed weaker umuC gene expression responses than the original TA1535/pSK1002 strain. 1,2-Epoxy-3-(4'-nitrophenoxy)propane, a known substrate for GST 5-5, was found to inhibit umuC induction caused by 1-bromo-2-chloroethane. These results indicate that this new tester NM5004 strain expressing a mammalian GST theta class enzyme may be useful for studies of environmental chems. proposed to be activated or inactivated by GST activity.

L7 ANSWER 17 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:987854 CAPLUS
DOCUMENT NUMBER: 124:77620
TITLE: A system to generate chromosomal mutations in Lactococcus lactis which allows fast analysis of targeted genes
AUTHOR(S): Law, Jean; Buist, Girbe; Haandrikman, Alfred; Kok, Jan; Venema, Gerard; Leenhouts, Kees
CORPORATE SOURCE: Dep. Genet., Univ. Groningen, Groningen, 9751 NN, Neth.
SOURCE: J. Bacteriol. (1995), 177(24), 7011-18
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A system for generating chromosomal insertions in lactococci is described. It is based on the conditional replication of lactococcal pWV01-derived Ori+ RepA- **vector** pORI19, contg. **lacZ**.alpha. and the multiple cloning site of pUC19. Chromosomal AluI fragments of Lactococcus lactis were cloned in pORI19 in RepA+ helper strain Escherichia coli EC101. The frequency of Campbell-type recombinants, following introduction of this **plasmid** bank into L. lactis (RepA-), was increased by combining the system with temp.-sensitive pWV01 deriv. pVE6007. Transformation of L. lactis MG1363 (pVE6007) with the pORI19 bank of lactococcal chromosomal fragments at the permissive temp. allowed replication of several copies of a recombinant **plasmid** from the bank within a cell because of the provision in trans of RepA-Ts from pVE6007. A temp. shift to 37.degree.C resulted in loss of pVE6007 and integration of the pORI19 derivs. at high frequencies. A bank of lactococcal mutants was made in this way and successfully screened for the presence of two mutations: one in the monocistronic 1.3-kb peptidoglycan hydrolase gene (acmA) and one in the hitherto uncharacterized maltose fermn. pathway. Reintroduction of pVE6007 into the Mal- mutant at 30.degree.C resulted in excision of the integrated **plasmid** and restoration of the ability to ferment maltose. The integration **plasmid** (pMAL) was rescued by using the isolated **plasmid** content of a restored Mal+ colony to transform E. coli EC101. Nucleotide

sequencing of the 564-bp chromosomal fragment in pMAL revealed an internal part of an open reading frame of which the translated product showed significant homol. with ATP-binding proteins MalK of E. coli, **Salmonella typhimurium**, and Enterobacter aerogenes and MsmK of Streptococcus mutans. This combined use of two types of conditional replicating pWV01-derived **vectors** represents a novel, powerful tool for chromosomal gene inactivation, targeting, cloning, and sequencing of the labeled gene.

L7 ANSWER 18 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:482647 CAPLUS
DOCUMENT NUMBER: 122:258442
TITLE: Development of high sensitive umu test system: rapid detection of genotoxicity of promutagenic aromatic amines by **Salmonella typhimurium** strain NM2009 possessing high O-acetyltransferase activity
AUTHOR(S): Oda, Yoshimitsu; Yamazaki, Hiroshi; Watanabe, Masahiko; Nohmi, Takehiko; Shimada, Tsutomu
CORPORATE SOURCE: Osaka Prefectural Institute of Public Health, 3-69 Nakamichi 1-chome, Higashinari-ku, Osaka, 537, Japan
SOURCE: Mutat. Res. (1995), 334(2), 145-56
CODEN: MUREAV; ISSN: 0027-5107
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A highly sensitive umu test system for the detection of carcinogenic/mutagenic arom. amines has been developed utilizing a new tester strain, **Salmonella typhimurium** NM2009, possessing an elevated O-acetyltransferase (O-AT) level. NM2009 was constructed by subcloning the bacterial O-AT gene into a **plasmid vector** pACYC184 and introducing the **plasmid** into the original strain **S. typhimurium** TA1535/pSK1002 harboring an umuC'-'**lacZ** fusion gene. The system is based on the ability of DNA-damaging agents (genotoxins) to induce umuC gene expression and monitored by measuring the cellular .**beta**.-**galactosidase** activity evoked by the fusion gene. Twenty-two arom. amine compds. including arylamines, aminoazo dyes, and heterocyclic arom. amines were tested for inducibility of DNA damage after metabolic activation by rat liver S9 in strain NM2009 and the sensitivity was compared with those of the parent strain TA1535/pSK1002 and the O-AT-defective strain NM2000. NM2009 had about 400 times higher O-AT activity than the parent strain. It was found that NM2009 was much more sensitive to arom. amines than other strains to induce umuC gene expression after metabolic activation; the chems. which were extremely sensitive in strain NM2009 include 2-aminoanthracene, 2-aminofluorene, 2-acetylaminofluorene, benzidine, 6-aminochrysene, 2,4-diaminotoluene, 2,6-diaminotoluene, 1-naphthylamine, o-tolidine, 3-methoxy-4-aminoazobenzene, o-aminoazotoluene, Glu-P-1, Trp-P-1, MeA.alpha.C, A.alpha.C, MeIQ, MeIQx, and IQ. In contrast, Trp-P-2 and PhIP showed almost similar sensitivities in three tester strains used in this study. These results suggest that strain NM2009 with high O-acetyltransferase activity is very useful to detect the genotoxic activities of potential mutagenic arom. amine compds., which require metabolic activation via the cytochrome P 450/acetyltransferase system.

L7 ANSWER 19 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:337613 CAPLUS
DOCUMENT NUMBER: 122:152707
TITLE: Expression of **LacZ** from the htrA, nirB and groE promoters in a Salmonella vaccine strain: Influence of growth in mammalian cells
AUTHOR(S): Everest, Paul; Frankel, Gad; Li, Jingli; Lund, Peter; Chatfield, Steven; Dougan, Gordon
CORPORATE SOURCE: Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, SW7 2AZ, UK
SOURCE: FEMS Microbiol. Lett. (1995), 126(1), 97-102
CODEN: FMLED7; ISSN: 0378-1097
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Attenuated Salmonella strains are currently being evaluated as live **vectors** for the delivery of heterologous antigens to the mammalian mucosal and systemic immune systems. An approach to improving the stability of heterologous antigen expression during vaccination is to drive expression of the foreign protein from promoters, e.g. nirB, that become activated when Salmonella enter the host. Salmonella strains were constructed that harbored similar multicopy **plasmids** encoding the **lacZ** gene. In each strain, **lacZ** expression was driven from either the nirB, htrA, or groE promoters. Expression of **LacZ** increased in all vaccine strains as they were shifted from conditions of low to high temp. In addn., expression of **lacZ** driven from the htrA and nirB promoters significantly increased when the Salmonella entered eukaryotic cells, including macrophages. Expression of **lacZ** from the groE promoter was significantly elevated in macrophages but not in cells derived from epithelia. These promoters may be useful for optimizing heterologous antigen expression within immune cells of the host.

L7 ANSWER 20 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:625701 CAPLUS
DOCUMENT NUMBER: 121:225701
TITLE: Method for detecting gene expression of internalized **Salmonella typhimurium** in macrophages
AUTHOR(S): Gracheck, Stephen J.; Wold, Sara A.
CORPORATE SOURCE: Exp. Therapy Dep., Warner Lambert Co., Ann Arbor, MI, USA
SOURCE: Dev. Plant Pathol. (1994), 3(Molecular Mechanisms of Bacterial Virulence), 267-80
CODEN: DPPAEF
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Expression of virulence factors is essential for microbial pathogenesis. We examd. the interaction of **S. typhimurium** and macrophages in an in vitro assay to assess the effects of antibiotics on bacterial viability and gene expression. The murine macrophage cell line J774A.1 and **S. typhimurium** strains TT12308 with a purG::**lacZ** (MudJ) fusion and x3181(pGTRO90) with a spvA::**lacZ** fusion were used. The TT12308 fusion is a constitutively expressed purine biosynthetic gene and the x3181(pGTRO90) fusion is an inducible

plasmid virulence gene necessary for systemic infection. The bacteria were exposed to ciprofloxacin and sparfloxacin, quinolone DNA gyrase inhibitors, and tetracycline either simultaneously to the macrophages, or following adherence and invasion. Exposure of the purG strain to ciprofloxacin at the time of adherence resulted in a dose dependent decrease in viable bacterial counts recovered internally from macrophages and ranged from a 0.5 to 2.2 log decrease at 1/2 to 16 .times. MIC (MIC = 0.003 .mu.g/mL), resp. Similarly purG expression, as measured by . **beta.-galactosidase** activity, decreased in a dose dependent manner, ranging from 25% to 75% of the control at 1/8 to 1/2 .times. MIC. No **.beta.-galactosidase** activity was seen above 1/2 .times. MIC. Ciprofloxacin, when added after adherence and invasion, did not decrease either bacterial viability or purG expression. Probenecid, a carrier-mediated transport inhibitor, added simultaneously with ciprofloxacin, had an additive effect on the decrease in recovery of viable bacteria. When sparfloxacin was added at the time of infection, a 2.5 log decrease occurred, and, when added 3 h post infection, only a 1.3 log decrease in viability of the purG strain was seen. In studies with the spvA strain treated in macrophages at the time of infection, sparfloxacin reduced the viability 4.5 logs and tetracycline had a bacteriostatic effect. When the drugs were added 3 h post infection, sparfloxacin reduced the no. of internalized organisms 3.3 logs, whereas tetracycline had little effect. In both treatment regimens, subinhibitory concns. of tetracycline induced a **.beta.-galactosidase** response in the spvA strain, whereas sparfloxacin had no effect at any concn. Induction was due to a tetracycline resistance promoter on the **vector** . This model can be utilized to evaluate the effect of antibiotics on the viability and gene expression of bacteria in macrophages.

L7 ANSWER 21 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:160947 CAPLUS
DOCUMENT NUMBER: 120:160947
TITLE: Use of incompatible **plasmids** to control expression of antigen by **Salmonella typhimurium** and analysis of immunogenicity in mice
AUTHOR(S): Ervin, Sean E.; Small, Parker A.; Gulig, Paul A.
CORPORATE SOURCE: Coll. Med., Univ. Florida, Gainesville, FL, 32610-0266, USA
SOURCE: Microb. Pathog. (1993), 15(2), 93-101
CODEN: MIPAEV; ISSN: 0882-4010
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Salmonella species have been investigated as live vaccine **vectors** because they are heat stable and can elicit humoral, cellular, and secretory immune responses. However, the expression of some foreign antigens is toxic to bacterial **vectors**. The authors therefore studied an approach for the controlled expression of antigen in **Salmonella typhimurium** wherein the antigen is not expressed in vitro but is expressed in vivo. A model antigen, **.beta.-galactosidase**, was expressed from the trc promoter on one **plasmid**, while repression was achieved by LacI expressed in trans from a second **plasmid**. The second repressor **plasmid** was incompatible with the expression **plasmid** encoding .

beta.-galactosidase. Loss by segregation of the repressor **plasmid** in vitro correlated with increased expression of .**beta.-galactosidase.** Oral inoculation of mice with salmonellae contg. both **plasmids** induced serum IgG but not nasal, salivary, or biliary IgA antibody to .**beta.-galactosidase.** Serum IgG as well as biliary IgA anti-**S. typhimurium** antibody, but not salivary or nasal IgA, were also detected. This salmonella **vector** system for the controlled expression of recombinant antigens may be of value for inducing systemic but not mucosal immunity to antigens that are toxic to bacterial **vectors.**

L7 ANSWER 22 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1993:553757 CAPLUS
DOCUMENT NUMBER: 119:153757
TITLE: Highly sensitive umu test system for the detection of mutagenic nitroarenes in **Salmonella typhimurium** NM3009 having high O-acetyltransferase and nitroreductase activities
AUTHOR(S): Oda, Yoshimitsu; Yamazaki, Hiroshi; Watanabe, Masahiko; Nohmi, Takehiko; Shimada, Tsutomu
CORPORATE SOURCE: Osaka Prefect. Inst. Public Health, Osaka, 537, Japan
SOURCE: Environ. Mol. Mutagen. (1993), 21(4), 357-64
CODEN: EMMUEG; ISSN: 0893-6692
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A highly sensitive umu test system for the detection of genotoxic activities of a variety of mutagenic nitroarenes has been developed using a new tester strain, **S. typhimurium** NM3009 having high O-acetyltransferase (O-AT) and nitroreductase (NR) activities. The NM3009 was constructed by subcloning both the O-AT and NR genes into **plasmid vector** pACYC184, and the resulting **plasmid** was introduced into the parent tester strain **S. typhimurium** TA1535/pSK1002 harboring an umcC'-'**lacZ** fusion gene. The induction of umuC gene expression could be monitored by measuring the cellular .**beta.-galactosidase** activity produced by fusion gene. The purpose of the study was to evaluate whether the newly developed strain NM3009 is highly sensitive toward nitroarene compds. The sensitivity of the strain NM3009 was compared with those of the parent TA1535/pSK1002 strain, the NR-overexpressing strain NM1011, the NR-deficient strain NM1000, the O-AT-overexpressing strain NM2009, and the O-AT-defective strain NM2000. The newly developed NM3009 strain had about 13-fold and 3-fold higher activities for N-AT and NR, resp., than the original **S. typhimurium** TA1535/pSK1002 strain. Among six strains tested, NM3009 showed the highest sensitivity toward such chems. as 1-nitronaphthalene, 2-nitrofluorene, 3,7-dinitrofluoranthene, 3-nitrofluoranthene, 5-nitroacenaphthene, 2-nitronaphthalene, 1-nitropyrene, 1,6-dinitropyrene, 3,9-dinitrofluoranthene, 4,4'-dinitrobiphenyl, 1,8-dinitropyrene, m-dinitrobenzene, 2,4-dinitrotoluene, and 1,3-dinitropyrene. The order of sensitivities to induce umuC gene expression toward a variety of nitroarenes was NM3009 > NM2009 > NM1011 > TA1535/pSK1002 > NM2000 > NM1000. Apparently, the newly developed tester strain NM3009 is of great use for the detection of genotoxic activities of

numerous carcinogenic and mutagenic chems. including nitroarenes, which require NR and(or) O-AT for the activation.

L7 ANSWER 23 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1992:77705 CAPLUS
DOCUMENT NUMBER: 116:77705
TITLE: Multiple mechanisms contribute to osmotic inducibility of proU operon expression in Escherichia coli: demonstration of two osmoresponsive promoters and of a negative regulatory element within the first structural gene
AUTHOR(S): Dattananda, C. S.; Rajkumari, K.; Gowrishankar, J.
CORPORATE SOURCE: Cent. Cell. Mol. Biol., Hyderabad, 500007, India
SOURCE: J. Bacteriol. (1991), 173(23), 7481-90
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Transcription of the proU operon in Escherichia coli is induced several hundredfold upon growth of cells in media of elevated osmolarity. A low-copy-no. promoter-cloning **plasmid vector**, with **lacZ** as the reporter gene, was used for assaying the osmoresponsive promoter activity of each of various lengths of proU DNA, generated by cloning of discrete restriction fragments and by an exonuclease III-mediated deletion approach. The results indicate that expression of proU in E. coli is directed from two promoters, one (P2) characterized earlier by other workers with the start site of transcription 60 nucleotides upstream of the initiation codon of the first structural gene (proV), and the other (P1) situated 250 nucleotides upstream of proV. Furthermore, a region of DNA within proV was shown to be involved in neg. regulation of proU transcription; phase Mu dII1681-generated lac fusions in the early region of proV also exhibited partial derepression of proU regulation, in comparison with fusions further downstream in the operon. Sequences around promoter P1, sequences around P2, and the promoter-downstream neg. regulatory element, resp., conferred approx. 5-, 8-, and 25-fold osmoresponsivity on proU expression. Within the region genetically defined to encode the neg. regulatory element, there is a 116-nucleotide stretch that is absolutely conserved between the proU operons of E. coli and **Salmonella typhimurium** and has the capability of exhibiting alternative secondary structure. Insertion of this region of DNA into each of two different **plasmid vectors** was assocd. with a marked redn. in the mean topol. linking no. in **plasmid** mols. isolated from cultures grown in high-osmolarity medium. It was proposed that this region of DNA undergoes reversible transition to an underwound DNA conformation under high-osmolarity growth conditions and that this transition mediates its regulatory effect on proU expression.

L7 ANSWER 24 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1992:52559 CAPLUS
DOCUMENT NUMBER: 116:52559
TITLE: Regulation of toxA and regA by the Escherichia coli fur gene and identification of a fur homolog in Pseudomonas aeruginosa PA103 and PA01
AUTHOR(S): Prince, R. W.; Storey, D. G.; Vasil, A. I.;

Vasil, M. L.
CORPORATE SOURCE: Health Sci. Cent., Univ. Colorado, Denver, CO, 80262, USA
SOURCE: Mol. Microbiol. (1991), 5(11), 2823-31
CODEN: MOMIEE; ISSN: 0950-382X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A multicopy **plasmid** contg. the E. coli fur gene was introduced into P. aeruginosa strain PA103C. This strain contains a toxA-**lacZ** fusion integrated into its chromosome at the toxA locus. **.beta.-Galactosidase** synthesis in this strain is regulated by iron, as is seen for exotoxin A prodn. **Beta-galactosidase** synthesis and exotoxin A prodn. in PA103C contg. multiple copies of E. coli fur was still represented in low iron conditions. The transcription of regA, a pos. regulator of toxA, was also found to be inhibited by multiple copies of the E. coli fur gene. In addn., the ability of PA103C contg. multiple copies of E. coli fur to produce protease was greatly reduced relative to PA103C contg. a **vector** control. A polyclonal rabbit serum contg. antibodies that recognize E. coli Fur was used to screen whole-cell exts. from Vibrio cholerae, Shigella flexneri, **Salmonella typhimurium**, and P. aeruginosa. All strains tested expressed a protein that was specifically recognized by the anti-Fur serum. These results suggest that Fur structure and function are conserved in a variety of distinct bacterial genera and that at least some of these different genera use this regulatory protein to control genes encoding virulence factors.

L7 ANSWER 25 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1991:625301 CAPLUS
DOCUMENT NUMBER: 115:225301
TITLE: Synthesis of the surface glycoprotein of rotavirus SA11 in the aroA strain of **Salmonella typhimurium** SL3261
AUTHOR(S): Salas-Vidal, E.; Plebanski, M.; Castro, S.; Perales, G.; Mata, E.; Lopez, S.; Arias, C. F.
CORPORATE SOURCE: Cent. Invest. Ing. Genet. Biotecnol., Univ. Nac. Auton. Mexico, Cuernavaca, 62271, Mex.
SOURCE: Res. Microbiol. (1990), 141(7-8), 883-6
CODEN: RMCREW; ISSN: 0923-2508
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The authors previously constructed **plasmid** pUMA93Z, which directs the synthesis in Escherichia coli of about 86% of the mature VP7 protein of rotavirus SA11 as a fusion product with **.beta.-galactosidase** (**.beta.gal**). Here, the attenuated aroA strain of **S. typhimurium** SL3261 (S. K. Hoiseth and B. A. D. Stocker, 1981) was used as a **vector** to deliver the VP7-**.beta.gal** recombinant polypeptide to the immune system of mice. **Plasmid** pUMA93Z was modified by insertion of the rop gene, which, in E. coli, has the effect of decreasing the copy no. of ColE1-related **plasmids**. Both pUMA93Z and the rop-modified **plasmid** were transfected into **S. typhimurium** SL3261. The recombinant strains carrying either **plasmid** synthesized the VP7-**.beta.gal** polypeptide, and it represented 1% of the total protein in bacteria

with pUMA93Z and 0.5% in bacteria contg. pUMA93Z-rop. Accordingly, lysates of bacteria carrying pUMA93Z had a 4-fold higher . **beta.-galactosidase** activity than those contg. the rop-modified **plasmid**. These results suggest that the rop protein is functional in the SL3261 Salmonella strain. The stability of the recombinant **plasmids** in SL3261 was detd. by serial broth culture in the absence of antibiotic pressure; after 40 generations, about 90% and 75% of the cells retained **plasmids** pUMA93Z and pUMA93Z-rop, resp. Mice immunized with **S. typhimurium** carrying **plasmid** pUMA93Z-rop developed antibodies against **S. typhimurium** lipopolysaccharide and **.beta. gal** but not to rotavirus SA11.

L7 ANSWER 26 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1991:2585 CAPLUS
DOCUMENT NUMBER: 114:2585
TITLE: Catalytic activities of human liver cytochrome P-450 IIIA4 expressed in Saccharomyces cerevisiae
AUTHOR(S): Brian, William R.; Sari, Marie Agnes; Iwasaki, Masahiko; Shimada, Tsutomu; Kaminsky, Laurence S.; Guengerich, F. Peter
CORPORATE SOURCE: Sch. Med., Vanderbilt Univ., Nashville, TN, 37232-0146, USA
SOURCE: Biochemistry (1990), 29(51), 11280-92
CODEN: BICHAW; ISSN: 0006-2960
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A human liver cytochrome P 450 (P 450) IIIA4 cDNA clone was inserted behind an alc. dehydrogenase promoter in the **plasmid vector** pAAH5 and expressed in Saccharomyces cerevisiae (D12 and AH22 strains). A cytochrome P 450 with typical spectral properties was expressed at a level of .apprx.8 .times. 105 mols./cell in either strain of yeast. The expressed P-450 IIIA4 had the same apparent monomeric Mr as the corresponding protein in human liver microsomes (P-450NF) and could be isolated from yeast microsomes. Catalytic activity of the yeast microsomes toward putative P 450 IIIA4 substrates was seen in the reactions supported by cumene hydroperoxide but was often lower and variable when supported by the physiol. donor NADPH. The catalytic activity of purified P 450 IIIA4 was also poor in some systems reconstituted with rabbit liver NADPH-P 450 reductase and best when both the detergent 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate and a lipid ext. (from liver or yeast microsomes) or L-.alpha.-1,2-dilauroyl-sn-glycero-3-phosphocholine were present. Under these conditions the expressed P 450 IIIA4 was an efficient catalyst for nifedipine oxidn., 6.beta.-hydroxylation of testosterone and cortisol, 2-hydroxylation of 17.beta.-estradiol and 17.alpha.-ethynylestradiol, N-oxygenation and 3-hydroxylation of quinidine, 16.alpha.-hydroxylation of dehydroepiandrosterone 3-sulfate, erythromycin N-demethylation, the 10-hydroxylation of (R)-warfarin, the formation of 9,10-dehydrowarfarin from (S)-warfarin, and the activation of aflatoxins B1 and G1, sterigmatocystin, 7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene (both + and - diastereomers), 3,4-dihydroxy-3,4-dihydrobenz[a]anthracene, 3,4-dihydroxy-3,4-dihydro-7,12-dimethylbenz[a]anthracene, 9,10-dihydroxy-9,10-dihydrobenzo[b]fluoranthene, 6-aminochrysene,

and tris(2,3-dibromopropyl) phosphate to products genotoxic in a **Salmonella typhimurium** TA1535/pSK1002 system where a chimeric umuC' '**lacZ plasmid** is responsive to DNA alkylation. Reaction rates were stimulated by 7,8-benzoflavone and inhibited by rabbit anti-P 450 IIIA (anti-P-450NF), troleandomycin, gestodene, cimetidine. Evidence was obtained that rates of redn. of ferric P 450 IIIA4 in yeast microsomes and the reconstituted systems are slow and at least partially responsible for the lower rates of catalysts seen in these systems (relative to liver microsomes). The results of these studies with a defined protein clearly demonstrate the ability of P 450 IIIA4 to catalyst regio- and stereoselective oxidns. with a diverse group of substrates, and this enzyme appears to be one of the most versatile catalysts in the P 450 family.

L7 ANSWER 27 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1990:17373 CAPLUS
DOCUMENT NUMBER: 112:17373
TITLE: Bacterial host cells and positive-selection **plasmids** for their stable transformation
INVENTOR(S): Curtiss, Ray, III
PATENT ASSIGNEE(S): Washington University, USA
SOURCE: PCT Int. Appl., 102 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 8903427	A1	19890420	WO 1988-US3496	19881006
W: AT, AU, BB, BG, BR, CH, DE, DK, FI, GB, HU, JP, KP, KR, LK, LU, MC, MG, MW, NL, NO, RO, SD, SE, SU				
RW: AT, BE, BJ, CF, CG, CH, CM, DE, FR, GA, GB, IT, LU, ML, MR, NL, SE, SN, TD, TG				
AU 8826111	A1	19890502	AU 1988-26111	19881006
AU 637969	B2	19930617		
EP 381706	A1	19900816	EP 1989-900028	19881006
EP 381706	B1	19950426		
R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
JP 04501351	T2	19920312	JP 1989-500124	19881006
AT 121785	E	19950515	AT 1989-900028	19881006
JP 11235191	A2	19990831	JP 1998-358165	19881006
US 5294441	A	19940315	US 1991-785748	19911107
US 5387744	A	19950207	US 1993-88394	19930707
US 5855879	A	19990105	US 1994-209542	19940310
US 5672345	A	19970930	US 1995-402308	19950310
US 5840483	A	19981124	US 1995-473926	19950607
US 5855880	A	19990105	US 1996-596732	19960205

PRIORITY APPLN. INFO.:	
US 1987-106072	19871007
US 1988-251304	19881003
US 1987-58360	19870604
US 1988-200934	19880601
JP 1989-500124	19881006
WO 1988-US3496	19881006
US 1990-612001	19901109
US 1991-785748	19911107

US 1992-975892	19921113
US 1992-990361	19921215
US 1994-209542	19940310
US 1995-402308	19950310

AB Bacterial strains from which an essential gene has been deleted perforce maintain a **plasmid** carrying a gene encoding a functional equiv. Methods for the generation of such mutants in the gene for aspartate semialdehyde dehydrogenase, essential for cell wall synthesis, in a no. of bacteria are described. Such **plasmids** can be used as expression **vectors** for vaccines, industrially useful enzymes, or as a selective agent to maintain the **plasmid** in a bacterial population released into the field. An asd mutant of **Salmonella typhimurium** was used as a host for expression **vectors** carrying an asd gene and the gene for the spaA antigen of Salmonella mutans. In these **vectors** the **plasmid** was stable in vitro, with a loss rate of 1%/bacterium/generation. When stability was tested in vivo in germ-free mice fed the recombinant organism it was found that all **S. typhimurium** recovered still carried the **plasmid** and the mice had developed salivary IgA and serum IgG antibodies to the protein.

L7 ANSWER 28 OF 35 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER:	1989:527907 CAPLUS
DOCUMENT NUMBER:	111:127907
TITLE:	Nucleotide sequence of the transcriptional control region of the osmotically regulated proU operon of **Salmonella typhimurium** and identification of the 5' endpoint of the proU mRNA
AUTHOR(S):	Overdier, David G.; Olson, Eric R.; Erickson, Bruce D.; Ederer, Martina M.; Csonka, Laszlo N.
CORPORATE SOURCE:	Dep. Biol. Sci., Purdue Univ., West Lafayette, IN, 47906, USA
SOURCE:	J. Bacteriol. (1989), 171(9), 4694-706 CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE:	Journal
LANGUAGE:	English

AB Southern blot anal. of 15 proU transposon insertions in **S. typhimurium** indicated that this operon is .gtoreq.3 kilobase pairs in length. The nucleotide sequence of a 1.5-kilobase-pair fragment that contains the transcriptional control region of the proU operon and the coding sequences specifying 290 amino acids of the first structural gene of the operon was detd. The predicted amino acid sequence of the product of this gene shows extensive similarity to the HisP, MalK, and other proteins that are inner membrane-assocd.8 components of binding protein-dependent transport systems. S1 mapping and primer extension anal. of the proU mRNAs revealed several species with different 5' ends. Two of these endpoints are sufficiently close to sequences that have weak similarities to the consensus -35 and -10 promoter sequences that they are likely to define 2 transcription start sites. However, the authors cannot rule out the possibility that some or all of the 5' endpoints detected arose as a result of the degrdn. of a longer mRNA. The expression of proU-**lacZ** operon fusions located on **plasmids** was normal in **S. typhimurium** , regardless of the **plasmid** copy no. The sequences

mediating normal, osmoregulated expression of the proU operon were shown by subcloning to be contained on an 815-base-pair fragment. A 350-base-pair subclone of this fragment placed onto a **lacZ** expression **vector** directed a high-level constitutive expression of **.beta.-galactosidase**, suggesting that there is a site for neg. regulation in the proU transcriptional control region which has been deleted in the construction of this **plasmid**.

L7 ANSWER 29 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1989:491724 CAPLUS
DOCUMENT NUMBER: 111:91724
TITLE: Method for preparing lamB protein-producing non-coliform prokaryotes, and use of these recombinants for introduction of desired genes into eukaryotic organisms such as plants
INVENTOR(S): Ludwig, Robert A.; De Vries, Gert E.
PATENT ASSIGNEE(S): University of California, USA
SOURCE: U.S., 10 pp. Cont.-in-part of U.S. Ser. No. 656,693, abandoned.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 4784952	A	19881115	US 1985-715124	19850321

PRIORITY APPLN. INFO.: US 1984-656693 19841001

AB A method for introducing a lamB gene into non-coliform prokaryotes is described. These transformants can be transformed with recombinant .lambda. phage contg. desired gene(s). An Escherichia coli mutant which expressed lamB constitutively was constructed. BglII-digested genomic DNA was ligated into a broad host range cosmid, and the resulting clone was used to prep. pTROY9, a tetracycline-resistance gene-contg. cosmid contg. the altered malK-lamB genome sequence. An E. coli (TRA+rec+) strain contg. pTROY9 and pKB242-12, a **plasmid** contg. a promoterless lamB-**lacZ** chimeric gene, was allowed to conjugate with Rhizobium meliloti. Growth of R. melitoti transformants on lactobionic acid selected for a recombinant **plasmid**, produced by recombination, in which the lamB-**lacZ** chimeric gene had acquired a promoter. The selection pressure was removed, which allowed the recombinant **plasmid**, a cointegrate, to dissoc. into **plasmids** pKB242-12 and an altered pTROY9. The altered **plasmid**, pTROY9', contained an altered lamB gene fused to a promoter functional in R. meliloti.

L7 ANSWER 30 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1989:491711 CAPLUS
DOCUMENT NUMBER: 111:91711
TITLE: Expression **vectors** for regulatable expression of heterologous genes in prokaryotes
INVENTOR(S): Schumacher, Guenther; Jarsch, Michael; Boos, Winfried
PATENT ASSIGNEE(S): Boehringer Mannheim G.m.b.H., Fed. Rep. Ger.
SOURCE: Ger. Offen., 16 pp.

CODEN: GWXXBX
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DE 3716957	A1	19881201	DE 1987-3716957	19870520
WO 8809373	A1	19881201	WO 1988-EP446	19880519
W: JP, US				
RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE				
JP 01501364	T2	19890518	JP 1988-504092	19880519
EP 316378	A1	19890524	EP 1988-904223	19880519
EP 316378	B1	19941102		
R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
US 6190906	B1	20010220	US 1989-300357	19890123

PRIORITY APPLN. INFO.: DE 1987-3716957 A 19870520
WO 1988-EP446 W 19880519

AB Expression **vectors** for regulatable expression of heterologous genes in prokaryotes comprise the promoter/operater region and the translation initiation signal of the mal operon, and, optionally, the signal sequence of the malB gene. Expression from this promoter/operator can be .apprx.100-fold decreased by the presence of catabolite repressors, e.g. glucose. Thus, an M13mp8 deriv. contg. the **Salmonella typhimurium** mal operon promoter/operator from pNM506 operably linked to the gene for mature endo-.beta.-N-acetylglucosaminidase H was constructed. Escherichia coli transformed with this **plasmid** produced .apprx.100 units of enzyme activity in LB medium. Upon addn. of 0.2% glucose, no activity was detectable.

L7 ANSWER 31 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1988:449337 CAPLUS
DOCUMENT NUMBER: 109:49337
TITLE: Molecular cloning, physical mapping and expression of the bet genes governing the osmoregulatory choline-glycine betaine pathway of Escherichia coli
AUTHOR(S): Andresen, Per A.; Kaasen, Inga; Styrvold, Olaf B.; Boulnois, Graham; Strom, Arne R.
CORPORATE SOURCE: Inst. Fish., Univ. Tromso, Tromso, N-9001, Norway
SOURCE: J. Gen. Microbiol. (1988), 134(6), 1737-46
CODEN: JGMIAN; ISSN: 0022-1287
DOCUMENT TYPE: Journal
LANGUAGE: English

AB An anal. of the bet genes governing the osmoregulatory choline-glycine betaine pathway of E. coli was performed. A 9 kb BamHI fragment, located 30 to 39 kb counterclockwise of the EcoRI site of **lacZ**, coded for all known Bet activities. The following genes were identified: the betA gene for choline dehydrogenase, the betB gene for betaine aldehyde dehydrogenase, and the betT gene or operon for high-affinity choline transport. The betB and the betT genes are named in this paper. The clockwise gene order was shown to be betA,B,T. Subcloning gave **plasmids** which expressed each of the three Bet activities sep. The cloned bet genes remained osmotically regulated, indicating the existence

of several osmotically regulated promoters in the bet region. **Salmonella typhimurium**, Which carried the bet region of E. coli in the broad-host-range **vector** pRK293, expressed the three Bet activities and displayed increased osmotic tolerance in the presence of choline.

L7 ANSWER 32 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1988:144327 CAPLUS
DOCUMENT NUMBER: 108:144327
TITLE: Cloning and characterization of the metC gene from **Salmonella typhimurium LT2**
AUTHOR(S): Park, Young M.; Stauffer, George V.
CORPORATE SOURCE: Dep. Microbiol., Univ. Iowa, Iowa City, IA, 52242, USA
SOURCE: Gene (1987), 60(2-3), 291-7
CODEN: GENED6; ISSN: 0378-1119
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The metC gene (encoding .beta.-cystathionase) of **S. typhimurium** was cloned into the **plasmid vectors** pACYC184 and pBR322. Genetic and biochem. expts. indicate that the region controlling metC gene expression is present on the cloned fragments. The location of the metC gene was detd. by insertional inactivation with transposons Tn5 and mini-Mu. The gene product was identified in a minicell system as a 49-kDa polypeptide. The direction of transcription and translation was detd. by correlating the orientation of mini-Mu insertions within the metC gene with the expression of the **lacZ** gene contained in mini-Mu.

L7 ANSWER 33 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1987:612942 CAPLUS
DOCUMENT NUMBER: 107:212942
TITLE: Use of .lambda. vehicles to isolate ompC-**lacZ** gene fusions in **Salmonella typhimurium LT2**
AUTHOR(S): Harkki, Anu; Karkku, Hanna; Palva, E. Tapio
CORPORATE SOURCE: Dep. Genet., Univ. Helsinki, Helsinki, SF-00100, Finland
SOURCE: MGG, Mol. Gen. Genet. (1987), 209(3), 607-11
CODEN: MGGEAE; ISSN: 0026-8925
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A novel **plasmid vector**, pAMH70 carrying both the lamB and nusA genes of Escherichia coli K12 was constructed. Introduction of this **plasmid** into **S. typhimurium LT2** renders this bacterium both sensitive to .lambda. adsorption and able to sustain growth and lysogenization by .lambda.. Using this strain as a recipient, stable gene fusions to the gene encoding a major outer membrane porin protein OmpC, were constructed with a .lambda. vehicle .lambda. placMu. To confirm the actual site of fusions, they were genetically mapped and transducing phages carrying the ompC-**lacZ** fusion were isolated and relysogenized. The fusions were also shown to be to ompC by their regulatory properties.

L7 ANSWER 34 OF 35 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1986:620175 CAPLUS
DOCUMENT NUMBER: 105:220175
TITLE: Expression of the SOS genes of Escherichia coli in **Salmonella typhimurium**
AUTHOR(S): Barbe, Jordi; Vericat, Joan Albert; Llagostera, Montserrat; Guerrero, Ricardo
CORPORATE SOURCE: Inst. Fundam. Biol., Auton. Univ. Barcelona, Bellaterra, Spain
SOURCE: Microbiologia (Madrid) (1985), 1(1-2), 77-87
CODEN: MICBE3; ISSN: 0213-4101
DOCUMENT TYPE: Journal
LANGUAGE: English
AB To lysogenize **S. typhimurium** by .lambda. phase, a region of 10.2 kb of E. coli DNA carrying the nusA gene was cloned in a **S. typhimurium** strain contg. a F'112 **plasmid** which codifies for the lamB region of E. coli. The strain of **S. typhimurium** obtained in this way, was lysogenized by .lambda.cInd0- phage harboring either a fusion between recA1 or sfiA genes of E. coli with the **lacZ** gene. Likewise, pSE143 **plasmid** with a umu C::**lacZ** fusion was introduced in **S. typhimurium**. Afterwards, induction of these SOS genes was studied. Results show that the basal transcription of both recA and sfiA genes of E. coli was higher in **S. typhimurium** than in E. coli. Nevertheless, induction of recA and sfiA genes by UV-irradn. and mitomycin C was higher in E. coli than in **S. typhimurium**. On the other hand, umuC gene of E. coli presents the same basal level of transcription in both E. coli and **S. typhimurium** species, although induction of this gene by UV-irradn. and mitomycin C [50-07-7] was higher in **S. typhimurium** than in E. coli. Therefore, **plasmid** pUA25 may be used to introduce, using the .lambda. phase as a **vector**, the SOS genes of E. coli in other bacterial species which may be useful to study the relationship between their resp. SOS systems.

L7 ANSWER 35 OF 35 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1985:499554 CAPLUS
DOCUMENT NUMBER: 103:99554
TITLE: Characterization of the **Salmonella typhimurium** mgl operon and its gene products
AUTHOR(S): Mueller, Norbert; Heine, Hans Georg; Boos, Winfried
CORPORATE SOURCE: Dep. Biol., Univ. Konstanz, Konstanz, D-7750, Fed. Rep. Ger.
SOURCE: J. Bacteriol. (1985), 163(1), 37-45
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In **S. typhimurium** and Escherichia coli the high-affinity galactose [59-23-4] transport system, which contains a periplasmic galactose-binding protein as an essential component, is encoded by the mgl genes. The entire mgl region of **S. typhimurium** is contained on a 6.3-kilobase EcoRI restriction fragment, which has been cloned into **plasmid vectors**. The extent of the mgl region on this fragment was detd. by Tn5 mutagenesis, examn. of **lacZ** fusions to mgl

genes, and subcloning smaller restriction fragments. Polyacrylamide gel electrophoresis of protein prepns. derived from strains carrying different **plasmids** was used to identify the mgl gene products. Thus, the mgl operon consists of 4 genes that form a single transcription unit: mglB, mglA, mglE, and mglC. The mglB gene codes for galactose-binding protein (33,000 daltons), mglA codes for a membrane-bound protein of 51,000 daltons, and mglC codes for a 29,000-dalton membrane protein. The mglE product was less well characterized. Its existence was inferred from a mglE-**lacZ** protein fusion located between mglA and mglC. In addn., the coupled transcription-translation in vitro system indicated that mglE codes for a 21,000-dalton protein.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO, TOXCENTER, PHIC, PHIN' ENTERED AT 10:00:24 ON 20 MAR 2002)
L11 132 S L6
L12 111 S L11 AND (CMV? OR PCMV? OR PLASMID)

L19 4C DCP REM L12 (71 DUPLICATES REMOVED)

L19 ANSWER 1 OF 40 WPIDS COPYRIGHT 2002 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2001-328017 [34] WPIDS
DOC. NO. CPI: C2001-100546
TITLE: New recombinant gut-colonizing microorganism, useful as vaccine component, comprises construct containing phoP, pagC or ompC gene promoter linked to nucleic acid encoding protein that induces immune response against pathogen.
DERWENT CLASS: B04 D16
INVENTOR(S): BULLIFENT, H L; TITBALL, R W
PATENT ASSIGNEE(S): (MINA) UK SEC FOR DEFENCE
COUNTRY COUNT: 93
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001019974	A2	20010322	(200134)*	EN	33

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

AU 2000070206 A 20010417 (200140)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001019974	A2	WO 2000-GB3402	20000906
AU 2000070206	A	AU 2000-70206	20000906

FILING DETAILS:

PATENT NO	KIND	PATENT NO

AU 2000070206 A Based on WO 200119974

PRIORITY APPLN. INFO: GB 2000-17000 20000712; GB 1999-21275 19990910

AN 2001-328017 [34] WPIDS

AB WO 200119974 A UPAB: 20010620

NOVELTY - A recombinant gut-colonizing microorganism (I) transformed with a construct (II) comprising P(phoP), P(pagC) or P(ompC) promoter, its fragment or variant which can **act** as a promoter, operably linked with a nucleic acid which encodes a protein, able to induce a protective immune response against an organism in a mammal, where (II) contains no further elements of phoP, pagC or ompC gene, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) enhancing expression of a desired protein at mucosal effector sites involves placing the protein to be expressed under the control of a promoter having a fully defined P(lac), P(phoP), P(pagC), P(ompC) (III) sequence of 71 (S1), 139 (S2), 715 (S3) or 371 (S4) nucleotides, respectively as given in the specification, its fragment or variant having the promoter activity, and causing expression in mucosal cells;

(2) (II) comprising P(phoP), P(pagC) or P(ompC) promoter, its fragment or variant which can **act** as promoter, operably linked with a nucleic acid which encodes a protein, able to induce a protective immune response against an organism in a mammal to which it is administered;

(3) a vaccine (IV) comprising (I); and

(4) use of (III) for the production of a vaccine comprising (I).

ACTIVITY - Antibacterial; antiviral.

MECHANISM OF ACTION - Vaccine.

The biological activity of (I) was tested in mice. Groups of 5 or 8 female BALB/c mice were immunized via intragastric (i.g.) intubation on days 0 and 14 with 1 multiply 10 to the power of 9 cells of the P(ompC)-F1, P(phop)-F1, P(pagC)-F1 or P(lac2)-F1 constructs, or the control **Salmonella typhimurium** strain, SL3261, in 0.1 ml of phosphate-buffered saline. On days 21, 28 and 98 mice were anesthetized and blood was collected. Blood was allowed to clot and serum was prepared stored at -20 deg. C until tested. After i.g. dosing with the recombinant Salmonella, mice in all groups developed immunoglobulin G (IgG) serum antibody to the carrier bacterium, which reached a maximum level 98 days after immunization. In the case of SL3261 expressing P(phoP)-F1, the onset of the immune response was delayed. All groups of mice developed IgG serum antibody to F1-antigen, which had reached a peak at day 28 and declined by day 98. There was no significant difference in the peak titers to F1-antigen induced by the different recombinant Salmonella. The ability of the different recombinant Salmonella to induce a mucosal antibody response after i.g. dosing was determined by measuring the levels of circulating IgA antibody to F1-antigen or the levels of IgA antibody to F1-antigen in gut or lung wash samples. The results indicated that SL3261 containing pP(phoP)-F1, pP(ac2)1-F1 or pP(pagC)-F1 **plasmids** all induced serum immunoglobulin A (IgA) antibody to F-antigen. The induction of circulating IgA to F1-antigen did not correlate with the presence of IgA to F1-antigen at mucosal surfaces. SL3261/pP(lac2)-F1 induced high levels of serum antibody to F1-antigen but IgA antibody to

F1-antigen was not detected in gut or lung wash samples. Only SL3261/pP(phoP)-F1 induced an IgA antibody response to F1-antigen in both the gut and the lung. Peyer's patches were also removed to determine the presence of F1- and Salmonella specific IgA producing cells in the gut. The results showed that the secretion of IgA against F1-antigen was greatest in Peyer's patch cells isolated from mice which had been immunized with SL3261 containing pP(pagC)-F1. Peyer's patch cells taken from mice which had been immunized with SL3261/pP(phoP)-F1 also produced IgA.

USE - Enhancing expression of a desired protein at mucosal effector sites (claimed). (I) is useful for delivering a variety of antigenic agents which can be used to induce a protective immune response against a wide range of pathogens such as Bacillus anthracis, Bordetella pertussis, Schistoma mansoni, herpes simplex virus, Mycobacterium tuberculosis etc. (III) is thus useful for preparation of vaccines for therapeutic or prophylactic uses.

ADVANTAGE - The three promoters (P(phoP), P(pagC) and P(ompC)) which are included in (I) are induced at different stages in the infection process, and hence at different sites in the body. This approach allows the induction of different immune responses which provide protection against pathogens which colonize different host cell compartments. The Salmonella vaccine **vector** system is ideally suited to the delivery of many vaccine antigens since the vaccine delivery mechanism accurately mimics the natural disease, entering the body via the gut.

Dwg.0/7

L19 ANSWER 2 OF 40 MEDLINE DUPLICATE 1

ACCESSION NUMBER: 2001394373 MEDLINE
DOCUMENT NUMBER: 21223403 PubMed ID: 11322824
TITLE: Construction and characterization of Streptococcus suis-Escherichia coli shuttle cloning **vectors**.
AUTHOR: Takamatsu D; Osaki M; Sekizaki T
CORPORATE SOURCE: Laboratory of Molecular Bacteriology, National Institute of Animal Health, 3-1-1 Kannondai, Tsukuba, Ibaraki, 305-0856, Japan.. p1013dt@niah.affrc.go.jp
SOURCE: PLASMID, (2001 Mar) 45 (2) 101-13.
Journal code: P8P; 7802221. ISSN: 0147-619X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AB037175; GENBANK-AB042426; GENBANK-AB042430; GENBANK-AB042431
ENTRY MONTH: 200107
ENTRY DATE: Entered STN: 20010716
Last Updated on STN: 20010716
Entered Medline: 20010712

AB pSSU1, a native **plasmid** of Streptococcus suis DAT1, was used to construct pSET-series shuttle **vectors**. In addition to the replication function of pSSU1, these **vectors** contain the multiple cloning sites and **lacZ'** gene from pUC19, which means that X-gal screening can be used to select recombinants in Escherichia coli. pSET1, pSET2, and pSET3 carry cat, spc, and both of these genes, respectively, as selectable markers. These **vectors** could be introduced into S. suis, E. coli, **Salmonella typhimurium**, S. pneumoniae, and S. equi

ssp. equi by electrotransformation. The recA gene was cloned from S. suis and sequenced, and this information was used in the construction of a recA mutant of S. suis. Transformation frequencies and/or **plasmid** stability of all pSET **vectors** tested were decreased in both S. suis and E. coli recA mutants compared with the parental strains. These results suggested that functional RecA protein improved the maintenance of pSET **vectors** in both S. suis and E. coli. Moreover, cloning of the functional S. suis recA gene into pSET2 and complementation analysis of the recA mutant were successful in S. suis but not in E. coli. These results showed that pSET **vectors** are useful tools for cloning and analyzing S. suis genes in S. suis strains directly. Copyright 2001 Academic Press.

L19 ANSWER 3 OF 40 JICST-EPlus COPYRIGHT 2002 JST
ACCESSION NUMBER: 1020030163 JICST-EPlus
TITLE: Construction of Streptococcus suis-Escherichia coli Shuttle **Vectors**.
AUTHOR: TAKAMATSU DAISUKE; OSAKI YOSHITO; SEKIZAKI TSUTOMU
CORPORATE SOURCE: Minist. of Agric., For. and Fish., Natl. Inst. of Anim. Health
SOURCE: Kachiku Eisei Kenkyu Seika Joho (Research Report of Animal Health), (2001) no. 14, pp. 25-26. Journal Code: J0532A (Fig. 3)
ISSN: 0916-1244
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Commentary
LANGUAGE: Japanese
STATUS: New
AB A native **plasmid** of Streptococcus suis DAT1, pSSU1, was used to construct pDAT series shuttle **vectors**. In addition to the replication function of pSSU1, these **vectors** contain the multiple cloning sites and **lacZ'** gene from pUC19, which means that X-gal screening can be used to select recombinants in Escherichia coli. pDAT1, pDAT2, and pDAT3 carry cat, spc, and both genes as selectable markers, respectively. These **vectors** can be introduced into S. suis, E. coli. **Salmonella typhimurium**, Streptococcus pneumoniae, and Streptococcus equi subsp. equi by electroporation. The recA gene was cloned from S. suis and sequenced. Then, an S. suis recA mutant strain was constructed via homologous recombination. Furthermore, cloning of a functional S. suis recA gene into pDAT2 and complementation analysis of the recA mutant were successful in S. suis, but not in E. coli. These results show that pDAT **vectors** are useful tools for cloning and analyzing S. suis genes in S. suis strains directly. (author abst.)

L19 ANSWER 4 OF 40 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2001205969 EMBASE
TITLE: Application of the Lux-Fluoro test as bioassay for combined genotoxicity and cytotoxicity measurements by means of recombinant **Salmonella typhimurium** TA1535 cells.
AUTHOR: Baumstark-Khan C.; Rode A.; Rettberg P.; Horneck G.
CORPORATE SOURCE: C. Baumstark-Khan, DLR, Institut fur Luft-/Raumfahrtmedizin, Strahlenbiologie, 51147 Koln, Germany. christa.baumstark-khan@dlr.de
SOURCE: Analytica Chimica Acta, (20 Jun 2001) 437/1 (23-30).

Refs: 37
ISSN: 0003-2670 CODEN: ACACAM
PUBLISHER IDENT.: S 0003-2670(01)00974-6
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
052 Toxicology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The Lux-Fluoro test is a bioassay, which coincidentally measures the cytotoxic and genotoxic potency of a given substance based on the receptor reporter principle. For genotoxicity testing, bioluminescence occurs as a response to the presence of DNA-damaging agents. It is brought about by the induction of the promoterless luxCDABFE genes of Photobacterium leiognathi as reporter component under the control of a strong SOS promoter as receptor component. At concentrations of DNA-damaging agents which only scarcely affect cell survival, a high level of light production is induced. For testing cytotoxic agents viability of the bacteria is included as a test parameter describing the cytotoxicity of the agents. For that reason, **Salmonella typhimurium** TA1535 cells transformed with the bacterial protein expression **vector** pGFPuv were employed. This **plasmid** controls green fluorescent protein (GFP) expression by the lac promoter in TA1535 cells constitutively. A panel of recombinant **S. typhimurium** strains carrying either the SOS-Lux-**plasmid** (TA1535-pPLS-1) or the fluorescence mediating lac-GFPuv **plasmid** (TA1535-pGFPuv) was used to record in parallel agents (mitomycin C, chloramphenicol, doxorubicin hydrochloride, bleomycin sulphate and hydrogen peroxide) that effect the genetic material conveying either genotoxic, cytotoxic or geno- and cytotoxic effects. The light and fluorescence transmissions of untreated and chemical-treated cells were measured in a microtiter plate reader and luminescence induction factors (F(i)) as well as fluorescence deduction factors (F(d)) were calculated for the genotoxic and cytotoxic potential of the applied agents. .COPYRGT. 2001 Elsevier Science B.V.

L19 ANSWER 5 OF 40 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2000:540419 BIOSIS
DOCUMENT NUMBER: PREV200000540419
TITLE: Secondary transient transfection of stably transfected endothelial cells.
AUTHOR(S): Forough, Reza (1); Hawker, James R., Jr.
CORPORATE SOURCE: (1) Department of Medical Physiology, Health Science Center, Texas A and M University, Rm. 350, Reynolds Medical Bldg, College Station, TX, 77843 USA
SOURCE: Biotechniques, (october, 2000) Vol. 29, No. 4, pp. 720-726. print.
ISSN: 0736-6205.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

L19 ANSWER 6 OF 40 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2000:478503 BIOSIS
DOCUMENT NUMBER: PREV200000478503
TITLE: Infection by bacterial pathogens expressing type III

secretion decreases luciferase activity: Ramifications for reporter gene studies.

AUTHOR(S): Savkovic, Suzana D.; Koutsouris, Athanasia; Wu, Gary; Hecht, Gail (1)

CORPORATE SOURCE: (1) Department of Medicine, Section of Digestive and Liver Disease, University of Illinois, 840 South Wood Street, CSB Room 704, Chicago, IL, 60612 USA

SOURCE: Biotechniques, (September, 2000) Vol. 29, No. 3, pp. 514-522. print.
ISSN: 0736-6205.

DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Pathogenic microbes influence gene regulation in eukaryotic hosts. Reporter gene studies can define the roles of promoter regulatory sequences. The effect of pathogenic bacteria on reporter genes has not been examined. The aim of this study was to identify which reporter genes are reliable in studies concerning host gene regulation by bacterial pathogens expressing type III secretory systems. Human intestinal epithelial cells, T84, Caco-2 and HT-29, were transfected with **plasmids** containing luciferase (luc), chloramphenicol acetyltransferase (CAT) or **beta-galactosidase** (**beta-gal**) as reporter genes driven by the inducible interleukin-8 (IL-8) or constitutively active simian virus 40 (SV40) promoter. Cells were infected with enteropathogenic E. coli or **Salmonella typhimurium**, and the reporter activity was assessed. Luc activity significantly decreased following infection, regardless of the promoter. The activity of recombinant luc was nearly ablated by incubation with either EPEC or Salmonella in a cell-free system. Activity was partially preserved by protease inhibitors, and immunoblot analysis showed a decreased amount and molecular weight of recombinant luc, suggesting protein degradation. Neither **beta-gal** nor CAT activity was altered by infection. Disruption of type III secretion prevented the loss of luc activity. We conclude that CAT or **beta-gal**, but not luc, can be used as reliable reporter genes to assess the impact of pathogenic microbes, especially those expressing type III secretion on host cell gene regulation.

L19 ANSWER 7 OF 40 MEDLINE DUPLICATE 2

ACCESSION NUMBER: 2001053874 MEDLINE
DOCUMENT NUMBER: 20395375 PubMed ID: 10941927

TITLE: Constitutively expressed phoP inhibits mouse-virulence of **Salmonella typhimurium** in an Spv-dependent manner.

AUTHOR: Matsui H; Kawakami T; Ishikawa S; Danbara H; Gulig P A

CORPORATE SOURCE: Laboratory of Infectious Diseases and Immunology, Center for Basic Research, The Kitasato Institute, Tokyo, Japan.. matsui-h@kitasato.or.jp

CONTRACT NUMBER: AI28421 (NIAID)
SOURCE: MICROBIOLOGY AND IMMUNOLOGY, (2000) 44 (6) 447-54. Journal code: MX7. ISSN: 0385-5600.
PUB. COUNTRY: Japan
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 200012
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001211

AB In **Salmonella typhimurium**, the transcription of several virulence genes including spvB is regulated by the PhoP/PhoQ regulatory system. To further examine the relationship between the PhoP/PhoQ and Spv systems for virulence in mice, we examined a non-polar phoP mutation combined with different virulence **plasmid** genotypes for effects on virulence of **S. typhimurium** in the mouse model. PhoP-/Spv+ and PhoP-/Spv- mutants were not detectably recovered from the spleens of subcutaneously or orally inoculated mice. The phoP gene constitutively expressed from the **lacZ** promoter of a low copy number **vector** (phoP(C)) only partially complemented the non-polar phoP mutation for mouse-virulence in both the Spv+ and Spv- backgrounds; both PhoP(C) strains exhibited virulence equal only to a PhoP+/Spv- strain. Interestingly, in a PhoP+ background, the phoP(C) gene reduced splenic infection of the Spv+ but not Spv- salmonellae after subcutaneous or oral inoculation compared with the PhoP+ parents. Additionally, the phoP(C) gene in an Spv+ background reduced the net growth of salmonellae in macrophages in vitro; phoP(C) in an Spv- background was without effect. These data suggest that the constitutive expression of the phoP gene attenuates the virulence of **S. typhimurium** in mice in an Spv-dependent manner.

L19 ANSWER 8 OF 40 WPIDS COPYRIGHT 2002 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2000-097572 [08] WPIDS
DOC. NO. NON-CPI: N2000-075395
DOC. NO. CPI: C2000-028342
TITLE: Identifying ligands useful as inhibitors for treating bacterial infection.
DERWENT CLASS: B04 D16 J04 S03
INVENTOR(S): BALGANESH, T; RAMACHANDRAN, V; SHARMA, U
PATENT ASSIGNEE(S): (ASTR) ASTRA AB; (ASTR) ASTRAZENECA AB
COUNTRY COUNT: 87
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9964866	A1	19991216	(200008)*	EN	37
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW					
AU 9946687	A	19991230	(200022)		
EP 1086377	A1	20010328	(200118)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9964866	A1	WO 1999-SE979	19990607

AU 9946687	A	AU 1999-46687	19990607
EP 1086377	A1	EP 1999-930078	19990607
		WO 1999-SE979	19990607

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9946687	A	Based on	WO 9964866
EP 1086377	A1	Based on	WO 9964866

PRIORITY APPLN. INFO: SE 1998-2573 19980717; IN 1998-1239 19980609

AN 2000-097572 [08] WPIDS

AB WO 9964866 A UPAB: 20000215

NOVELTY - Identifying ligands to the sigma70 subunit of bacterial RNA polymerase, involves adding test compounds to the subunit (or the anti-sigma binding region of the subunit), and a fusion protein of an anti-sigma70 factor (I) or (II) of bacteriophage T4. Competitive binding of the test ligand and anti-sigma70 factor to the subunit is then determined.

ACTIVITY - Antibacterial.

MECHANISM OF ACTION - Bacterial sigma70 inhibitor.

USE - The method is useful for in vitro screening of peptide/chemical libraries for compounds that mimic the anti-sigma70 factor, in order to inhibit the binding of core RNA polymerase and sigma70 subunits. These compounds may be useful for treating bacterial infections, including conditions caused by Mycobacterium tuberculosis, **Salmonella typhimurium**, E. coli and Bacillus subtilis.

ADVANTAGE - The fusion protein facilitates identification of ligands by the method.

DESCRIPTION OF DRAWING(S) - The drawing shows a genetic map of the **plasmid vector** pARC8112

Dwg.1/8

L19 ANSWER 9 OF 40 WPIDS COPYRIGHT 2002 DERWENT INFORMATION LTD

ACCESSION NUMBER:	1999-215027 [18] WPIDS
DOC. NO. CPI:	C1999-063360
TITLE:	Nucleic acid from Borrelia burgdorferi encoding virulence associated protein P13.
DERWENT CLASS:	B04 D16
INVENTOR(S):	BERGSTROEM, S
PATENT ASSIGNEE(S):	(SYMB-N) SYMBICOM AB
COUNTRY COUNT:	83

PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9912960	A2	19990318	(199918)*	EN	117
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW					
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW					
AU 9888811	A	19990329	(199932)		
EP 1012269	A2	20000628	(200035)	EN	

R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9912960	A2	WO 1998-IB1424	19980904
AU 9888811	A	AU 1998-88811	19980904
EP 1012269	A2	EP 1998-940504	19980904
		WO 1998-IB1424	19980904

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9888811	A	Based on	WO 9912960
EP 1012269	A2	Based on	WO 9912960

PRIORITY APPLN. INFO: US 1997-59036P 19970916; DK 1997-1041 19970910

AN 1999-215027 [18] WPIDS

AB WO 9912960 A UPAB: 19990511

NOVELTY - Isolated nucleic acid (I) that:

(i) encodes a polypeptide fragment (II) immunologically reactive with rabbit polyclonal antibody raised against a 13 kDa polypeptide of Borrelia burgdorferi B313, but not reactive with most proteins from other spirochaetes, and/or

(ii) hybridizes under stringent conditions to specific nucleic acid sequences but not to genomic DNA from most other spirochaetes.

DETAILED DESCRIPTION - The rabbit antiserum is recognized by a protein encoded by a 759 bp genomic sequence (S1), and (I) hybridizes with (S1) or with 862 bp (S2) or 749 bp (S3) sequences. They do not react with proteins/genomic DNA from at least 95% of spirochaetes randomly selected from Borrelia hermsii, Borrelia crocidurae, Borrelia anserina and Borrelia hispanica.

All sequences are given in the specification.

INDEPENDENT CLAIMS are also included for the following:

(1) polypeptide (IIa) containing amino acids (aa) 1-167 of the 179 aa protein encoded by (S1), optionally in lipidated form;

(2) fusion proteins that include (IIa);

(3) non-borrelial **vector** containing (I);

(4) transformed cells containing this **vector**;

(5) stable cell lines producing (IIa), containing the **vector** of (3);

(6) vaccines containing (IIa);

(7) live vaccines comprising non-pathogenic microorganisms able to express (IIa);

(8) vaccines containing (I) or the **vector** of (3);

(9) diagnostic compositions containing (IIa), (I) or primers for amplification of (I) for determination of B. burgdorferi sensu lato; and

(10) a diagnostic kit comprising (II), (I) and primers for amplification of (I).

ACTIVITY - Antibacterial.

MECHANISM OF ACTION - Induction of specific immune response against B. burgdorferi sensu lato.

USE - (I), (II) and the transformed cells are useful in vaccines to protect against infection by B. burgdorferi sensu lato.

(I), (II) and antibodies raised against (II) are used to diagnose such infections, in standard immunoassays or amplification/hybridization tests. (I) are also used to produce recombinant (II).

ADVANTAGE - The 13 kD protein is involved in virulence and is highly conserved within B. burgdorferi sensu lato, but is absent from other Borrelia species (e.g. those responsible for relapsing fever or avain borrelosis). It should provide a more specific and sensitive antibody response, and diagnosis, compared with use of whole bacteria as antigen.

Dwg.0/9

L19 ANSWER 10 OF 40 MEDLINE DUPLICATE 3

ACCESSION NUMBER: 1999426805 MEDLINE
DOCUMENT NUMBER: 99426805 PubMed ID: 10496887
TITLE: Use of in vivo-regulated promoters to deliver antigens from attenuated Salmonella enterica var. Typhimurium.
AUTHOR: Dunstan S J; Simmons C P; Strugnell R A
CORPORATE SOURCE: Department of Microbiology and Immunology, The University of Melbourne, Parkville, Victoria 3052, Australia.. s.j.dunstan@ic.ac.uk
SOURCE: INFECTION AND IMMUNITY, (1999 Oct) 67 (10) 5133-41. Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199910
ENTRY DATE: Entered STN: 19991026
Last Updated on STN: 19991026
Entered Medline: 19991014

AB This study describes the construction and analysis of three in vivo-inducible promoter expression **plasmids**, containing pnirB, ppagC, and pkatG, for the delivery of foreign antigens in the DeltaaroAD mutant of Salmonella enterica var. Typhimurium (hereafter referred to as **S. typhimurium**). The reporter genes encoding **beta-galactosidase** and firefly luciferase were used to assess the comparative levels of promoter activity in **S. typhimurium** in vitro in response to different induction stimuli and in vivo in immunized mice. It was determined that the ppagC construct directed the expression of more **beta-galactosidase** and luciferase in **S. typhimurium** than the pnirB and pkatG constructs, both in vitro and in vivo. The gene encoding the C fragment of tetanus toxin was expressed in the aroAD mutant of **S. typhimurium** (BRD509) under the control of the three promoters. Mice orally immunized with attenuated **S. typhimurium** expressing C fragment under control of the pagC promoter [BRD509(pKK/ppagC/C frag)] mounted the highest tetanus toxoid-specific serum antibody response. Levels of luciferase expression in vivo and C-fragment expression in vitro from the pagC promoter appeared to be equivalent to if not lower than the levels of expression detected with the constitutive trc promoter. However, mice immunized with BRD509(pKK/ppagC/C frag) induced significantly higher levels of tetanus toxoid-specific antibody than BRD509(pKK/C frag)-immunized mice, suggesting that the specific location of foreign antigen expression may be important for immunogenicity.

Mutagenesis of the ribosome binding sites (RBS) in the three promoter/C fragment expression **plasmids** was also performed. Despite optimization of the RBS in the three different promoter elements, the expression levels in vivo and overall immunogenicity of C fragment when delivered to mice by attenuated **S. typhimurium** were not affected. These studies suggest that in vivo-inducible promoters may give rise to enhanced immunogenicity and increase the efficacy of **S. typhimurium** as a vaccine **vector**.

L19 ANSWER 11 OF 40 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 1999286196 MEDLINE
DOCUMENT NUMBER: 99286196 PubMed ID: 10357791
TITLE: Role of human N-acetyltransferases, NAT1 or NAT2, in genotoxicity of nitroarenes and aromatic amines in **Salmonella typhimurium** NM6001 and NM6002.
AUTHOR: Oda Y; Yamazaki H; Shimada T
CORPORATE SOURCE: Osaka Prefectural Institute of Public Health, 3-69 Nakamichi 1-chome, Higashinari-ku, Osaka 537-0025, Japan.
SOURCE: CARCINOGENESIS, (1999 Jun) 20 (6) 1079-83.
Journal code: C9T; 8008055. ISSN: 0143-3334.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199907
ENTRY DATE: Entered STN: 19990730
Last Updated on STN: 19990730
Entered Medline: 19990722

AB Human NAT1 and NAT2 genes were subcloned into pACYC184 **vector** and the **plasmids** thus obtained were introduced into **Salmonella typhimurium** O-acetyltransferase-deficient strain NM6000 (TA1538/1, 8-DNP/pSK1002), establishing new strains NM6001 and NM6002, respectively. We compared the sensitivities of these two strains with those of NM6000 towards carcinogenic nitroarenes and aromatic amines in the SOS/umu response. The induction of umuC gene expression by these chemicals in the presence and absence of the S9 fraction was assayed by measuring the cellular **beta-galactosidase** activity expressed by the umuC"**lacZ** fusion gene in the tester strains. 2-Nitrofluorene and 2-aminofluorene induced umuC gene expression more strongly in the NM6001 strain than in the NM6002 strain. In contrast, induction of umuC gene expression by 1, 8-dinitropyrene, 6-aminochrysene and 2-amino-3,5-dimethylimidazo[4, 5-f]quinoline was weaker in the NM6001 strain than in the NM6002 strain. 1-Nitropyrene, 2-amino-6-methyl-dipyrido[1,2-a:3', 2'-d]imidazole, 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole, 3-amino-1-methyl-5H-pyrido[4,3-b]indole, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine and 2-amino-3-methyl-9H-pyrido[2,3-b]indole were found to induce umuC gene expression at similar extents in both strains. These results suggest that the newly developed strains can be employed for the studies on mechanisms of genotoxicity of a variety of nitroarenes and aromatic amines, along with the assessment of cancer risk to humans.

L19 ANSWER 12 OF 40 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:274731 BIOSIS
DOCUMENT NUMBER: PREV199900274731
TITLE: Identification of a cis-acting regulatory sequence responsible for the repression of brnQ in **Salmonella typhimurium**.
AUTHOR(S): Ohnishi, Kuniharu (1); Matsubara, Keiko; Hattori, Yoshihiko; Sadanari, Hidetaka; Yamada, Rie; Fukuda, Shizuo
CORPORATE SOURCE: (1) Department of Microbiology, Faculty of Pharmaceutical Sciences, Hokuriku University, Kanazawa, 920-1181 Japan
SOURCE: Biochimica et Biophysica Acta, (May 14, 1999) Vol. 1445, No. 2, pp. 196-206.
ISSN: 0006-3002.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB brnQ is the gene encoding the LIV-II transport system for branched-chain amino acids in **Salmonella typhimurium**. The expression of the gene is transcriptionally repressed by an excess of glycyl-L-leucine added to the bacterial culture. To investigate the mechanism of regulation, we constructed brnQ-**lacZ** translational fusions with various deletions upstream from the promoter of brnQ, and examined the effects of the deletions on the regulation. We found a cis-acting region, 5 -GTGTTTTA-3 , for the repression of brnQ expression, which was located 94 base pairs upstream from the transcription start site. Removal of the sequence resulted in derepression of brnQ. Two homologous sequences were found 45 base pairs downstream and 42 base pairs upstream from the sequence. We designated these sequences as O1, O2, and O3, in the order from the sequence proximal to the promoter to that distal to the promoter, respectively. The gleR1 mutation, which we reported previously to be a regulatory mutation enhancing transcription of brnQ, was a G-to-T transversion in the O1 sequence 50 base pairs upstream from the transcription start site. Insertion of five nucleotides between O1 and O2 resulted in derepression of brnQ. Further insertion of five nucleotides did not restore the original regulation of brnQ, indicating the importance of the proper spacing of these sequences. We also showed that the protein product of livS, the gene responsible for regulation of the LIV-I transport system, may bind to the O2 sequence. Furthermore, LivS was shown to be an allele of Lrp based on complementation experiments.

L19 ANSWER 13 OF 40 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 2000063614 MEDLINE
DOCUMENT NUMBER: 20063614 PubMed ID: 10594975
TITLE: Introduction of protein or DNA delivered via recombinant **Salmonella typhimurium** into the major histocompatibility complex class I presentation pathway of macrophages.
AUTHOR: Catic A; Dietrich G; Gentschev I; Goebel W; Kaufmann S H; Hess J
CORPORATE SOURCE: Department of Immunology, University Clinics Ulm, D-89070 Ulm, Germany.
SOURCE: Microbes Infect, (1999 Feb) 1 (2) 113-21.
Journal code: DJ1; 100883508. ISSN: 1286-4579.

PUB. COUNTRY: France
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200007
ENTRY DATE: Entered STN: 20000810
Last Updated on STN: 20000810
Entered Medline: 20000727

AB Recombinant (r) **Salmonella typhimurium** aroA strains which display the hen egg ovalbumin OVA(257-264) peptide SIINFEKL in secreted form were constructed. In addition, attenuated rS. typhimurium pcDNA-OVA constructs harbouring a eukaryotic expression **plasmid** encoding complete OVA were used to introduce the immunodominant OVA(257-264) epitope into the major histocompatibility complex (MHC) class I presentation pathway. Both modes of antigen delivery (DNA and protein) by Salmonella vaccine carriers stimulated OVA(257-264)-specific CD8 T-cell hybridomas. An in vitro infection system was established that allowed both rSalmonella carrier devices to facilitate MHC class I delivery of OVA(257-264) by coexpression of **listeriolysin** (**Hly**) or by coinfection with rS. typhimurium Hlys (Hess J., Gentschev I., Miko D., Welzel M., Ladel C., Goebel W., Kaufmann S.H.E., Proc. Natl. Acad. Sci. USA 93 (1996) 1458-1463). Coexpression of **Hly** and coinfection with rS. typhimurium Hlys slightly improved MHC class I processing of OVA. Our data provide further evidence for the feasibility of attenuated, **Hly**-expressing rS. typhimurium carriers secreting heterologous antigens or harbouring heterologous DNA as effective vaccines for stimulating CD8 T cells in addition to CD4 T cells.

L19 ANSWER 14 OF 40 TOXCENTER COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:202358 TOXCENTER
COPYRIGHT: Copyright 2002 ACS
DOCUMENT NUMBER: CA12925329695D
TITLE: An attenuated strain of **Salmonella typhimurium** carrying eukaryotic expression constructs for use in oral vaccines
AUTHOR(S): Darji, Ayub; Guzman, Carlos; Timmis, Kenneth; Wehland, Jurgen; Weiss, Siegfried; Gerstel, Birgit; Chakraborty, Trinad; Wachholz, Petra
CORPORATE SOURCE: ASSIGNEE: Gesellschaft Fur Biotechnologische Forschung m.b.H.
PATENT INFORMATION: WO 9848026 A1 29 Oct 1998
SOURCE: (1998) PCT Int. Appl., 52 pp.
CODEN: PIXXD2.
COUNTRY: GERMANY, FEDERAL REPUBLIC OF
DOCUMENT TYPE: Patent
FILE SEGMENT: CAPLUS
OTHER SOURCE: CAPLUS 1998:709195
LANGUAGE: English
ENTRY DATE: Entered STN: 20011116
Last Updated on STN: 20011116

AN 1998:202358 TOXCENTER
CP Copyright 2002 ACS
AB An attenuated strain of **Salmonella typhimurium** has been developed for use as a vehicle for oral genetic immunization. Eukaryotic expression **vectors** contg. the genes for .**beta.-galactosidase**, or truncated

Devi, S.
09/419545

20mar02 10:43:43 User219783 Session D1806.1

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Derwent announces file enhancements. Please see HELP NEWS 357.
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S1 58078 (BETA OR B)(W)(GAL OR GALACTOSIDASE) OR LISTERIOLYSIN OR L-ISTERIO(W)LYSIN OR HLY OR ACTA OR ACT(W)A OR LACZ OR LAC(W)Z

S12 912 ATTENUAT?(5N)((SALMONEL? OR S)(W)TYPHI? OR TY21A OR TY(W)2-1A OR SL7207 OR SL(W)7207 OR 33275 OR LT2 OR LT(W)2)
S13 61 S1 AND S12
S14 37 S13 AND VECTOR? ?
S15 42 S13 AND (PLASMID? ? OR CMV? OR PCMV?)
S16 45 S14 OR S15
S17 38 RD (unique items)
>>>No matching display code(s) found in file(s): 65, 113

17/3,AB/1 (Item 1 from file: 144)
DIALOG(R)File 144:Pascal
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14743707 PASCAL No.: 00-0421162
Constitutively expressed phoP inhibits mouse-virulence of Salmonella typhimurium in an Spv-dependent manner
MATSUI H; KAWAKAMI T; ISHIKAWA S; DANBARA H; GULIG P A
Laboratory of Infectious Diseases and Immunology, Center for Basic Research, The Kitasato Institute, Minato-ku, Tokyo 108-8642, Japan; Department of Microbiology, School of Pharmaceutical Sciences, Kitasato University, Minato-ku, Tokyo 108-8641, Japan; Department of Molecular Genetics and Microbiology, University of Florida College of Medicine, Gainesville, FL 32610-0266, United States
Journal: Microbiology and immunology, 2000, 44 (6) 447-454
Language: English
In Sahnonella typhimurium, the transcription of several virulence genes including spvB is regulated by the PhoP/PhoQ regulatory system. To further

- Key terms

examine the relationship between the PhoP/PhoQ and Spv systems for virulence in mice, we examined a non-polar phoP mutation combined with different virulence *plasmid"** genotypes for effects on virulence of S. typhimurium in the mouse model. PhoP SUP - /Spv SUP + and PhoP SUP - / Spv SUP - mutants were not detectably recovered from the spleens of subcutaneously or orally inoculated mice. The phoP gene constitutively expressed from the *lacZ"** promoter of a low copy number *vector"** (phoP SUP c) only partially complemented the non-polar phoP mutation for mouse-virulence in both the Spv SUP + and Spv SUP - backgrounds; both PhoP SUP c strains exhibited virulence equal only to a PhoP SUP + /Spv SUP - strain. Interestingly, in a PhoP SUP + background, the phoP SUP c gene reduced splenic infection of the Spv SUP + but not Spv SUP - salmonellae after subcutaneous or oral inoculation compared with the PhoP SUP + parents. Additionally, the phoP SUP c gene in an Spv SUP + background reduced the net growth of salmonellae in macrophages in vitro; phoP SUP c in an Spv SUP - background was without effect. These data suggest that the constitutive expression of the phoP gene *attenuates"** the virulence of *S"**. *typhimurium"** in mice in an Spv-dependent manner.

17/3,AB/2 (Item 2 from file: 144)
DIALOG(R)File 144:Pascal

14686218 PASCAL No.: 00-0360941
Oral delivery of DNA vaccines using *attenuated"** *Salmonella"** *typhimurium"** as carrier
New Approaches to Bacterial Vaccine Development: 3rd International Meeting, 19-22 May 1999, Munich, Germany
DARJI A; ZUR LAGE S; GARBE A I; CHAKRABORTY T; WEISS S
VON SPECHT Bernd-Ulrich, ed
Molecular Immunology, GBF-National Research Center for Biotechnology, Mascheroder Weg I, 38124 Braunschweig, Germany; Institute for Medical Microbiology, University Giessen, Frankfurter Strasse 107, 35392 Giessen, Germany
Chirurgische Universitaetsklinik, Chirurgische Forschung, Freibug, Germany
International Meeting on New Approaches to Bacterial Vaccine Development, 3 (Munich DEU) 1999-05-19
Journal: FEMS immunology and medical microbiology, 2000, 27 (4) 341-349
Language: English
The efficacious delivery of eukaryotic expression *plasmids"** to inductive cells of the immune system constitutes a key prerequisite for the generation of effective DNA vaccines. Here, we have explored the use of bacteria as vehicles to orally deliver expression *plasmids"**. *Attenuated"** *Salmonella"** *typhimurium"** aroA harbouring eukaryotic expression *plasmids"** that encoded virulence factors of Listeria monocytogenes were administered orally to BALB/c mice. Strong cytotoxic and helper T cell responses as well as antibody production were elicited even after a single administration. Mice immunised four times with Salmonella that carried a eukaryotic expression *plasmid"** encoding the secretory listerial protein *listeriolysin"** were protected against a subsequent lethal challenge with this pathogen. A single dose was already partially protective. The efficiency of this vaccination procedure was due to transfer of the expression *plasmid"** from the bacterial carrier to the mammalian host. Evidence for such an event could be obtained in vivo and in

vitro. Expression of the desired antigen in various lymphoid tissues was already detectable 1 day after administration of the DNA vaccine and persisted for at least 1 month in spleen and mesenteric lymph nodes. Induction of cytotoxic and helper T cell responses was observed in all mouse strains tested including outbred strains whereas antibodies were mainly detected in BALB/c. Furthermore, we could show that immunogenicity could be improved by increasing the invasiveness of the bacterial carrier.

17/3,AB/3 (Item 3 from file: 144)
DIALOG(R)File 144:Pascal

14289729 PASCAL No.: 99-0495204

Use of in vivo-regulated promoters to deliver antigens from attenuated Salmonella enterica var. typhimurium

DUNSTAN S J; SIMMONS C P; STRUGNELL R A

Department of Microbiology and Immunology, The University of Melbourne, Parkville, Victoria 3052, United States; Co-Operative Research Centre for Vaccine Technology, The University of Melbourne, Parkville, Victoria 3052, Australia

Journal: Infection and immunity, 1999, 67 (10) 5133-5141

Language: English

This study describes the construction and analysis of three in vivo-inducible promoter expression *plasmids"**, containing pnirB, ppagC, and pkatG, for the delivery of foreign antigens in the DELTA aroAD mutant of Salmonella enterica var. Typhimurium (hereafter referred to as S. typhimurium). The reporter genes encoding 13-galactosidase and firefly luciferase were used to assess the comparative levels of promoter activity in S. typhimurium in vitro in response to different induction stimuli and in vivo in immunized mice. It was determined that the ppagC construct directed the expression of more *beta"** -*galactosidase"** and luciferase in S. typhimurium than the pnirB and pkatG constructs, both in vitro and in vivo. The gene encoding the C fragment of tetanus toxin was expressed in the aroAD mutant of S. typhimurium (BRD509) under the control of the three promoters. Mice orally immunized with *attenuated"** *S"**. *typhimurium"** expressing C fragment under control of the pagC promoter (BRD509(pKK/ppagC/C frag)) mounted the highest tetanus toxoid-specific serum antibody response. Levels of luciferase expression in vivo and C-fragment expression in vitro from the pagC promoter appeared to be equivalent to if not lower than the levels of expression detected with the constitutive trc promoter. However, mice immunized with BRD509(pKK/ppagC/C frag) induced significantly higher levels of tetanus toxoid-specific antibody than BRD509(pKK/C frag)-immunized mice, suggesting that the specific location of foreign antigen expression may be important for immunogenicity. Mutagenesis of the ribosome binding sites (RBS) in the three promoter/C fragment expression *plasmids"** was also performed. Despite optimization of the RBS in the three different promoter elements, the expression levels in vivo and overall immunogenicity of C fragment when delivered to mice by *attenuated"** *S"**. *typhimurium"** were not affected. These studies suggest that in vivo-inducible promoters may give rise to enhanced immunogenicity and increase the efficacy of S. typhimurium as a vaccine *vector"**.

17/3,AB/4 (Item 4 from file: 144)
DIALOG(R)File 144:Pascal

14050938 PASCAL No.: 99-0241214
Pathogenicity island 2 mutants of Salmonella typhimurium are efficient carriers for heterologous antigens and enable modulation of immune responses
MEDINA E; PAGLIA P; NIKOLAUS T; MULLER A; HENSEL M; GUZMAN C A
Department of Microbial Pathogenicity and Vaccine Research, Division of Microbiology, GBF-National Research Centre for Biotechnology, 38124 Braunschweig, Germany; Experimental Immunotherapy and Gene Therapy Unit, Istituto Nazionale per Lo Studio e la Cura dei Tumori, 20133 Milano, Italy; Lehrstuhl fuer Bakteriologie, Max von Pettenkofer-Institut fuer Hygiene und Medizinische Mikrobiologie, 80336 Munich, Germany
Journal: Infection and immunity, 1999, 67 (3) 1093-1099
Language: English
The potential use as vaccine delivery system of Salmonella typhimurium strains harboring defined mutations in the sseC (HH104) and sseD (MvP101) genes, which encode putative effector proteins of the type III secretion system of Salmonella pathogenicity island 2, was evaluated and compared with that of the well-characterized aroA mutant strain SL7207 by using *beta"** -*galactosidase"** (*beta"** -*Gal"**) as a model antigen. When orally administered to immune-competent or gamma interferon-deficient (IFN-gamma SUP - SUP / SUP -) BALB/c mice, both mutants were found to be highly attenuated (50% lethal dose, >10 SUP 9 bacteria). Both strains were also able to efficiently colonize and persist in Peyer's patches. Immunization with HH104 and MvP101 triggered *beta"** -*Gal"**-specific serum and mucosal antibody responses equivalent to or stronger than those observed in SL7207-immunized mice. Although immunoglobulin G2 (IgG2) serum antibodies were dominant in all groups, IgG1 was also significantly increased in mice vaccinated with MvP101 and SL7207. Comparable *beta"** -*Gal"**-specific IgA and IgG antibodies were detected in intestinal lavages from mice immunized with the different strains. Antigen-specific CD4 SUP + T-helper cells were generated after vaccination with all vaccine prototypes; however, responses were significantly more efficient when HH104 and MvP101 were used ($P < 0.05$). Significantly higher levels of IFN- gamma were produced by restimulated spleen cells from mice immunized with HH104 than from those vaccinated with the MvP101 or SL7207 derivatives ($P <= 0.05$). Interestingly, the three strains induced major histocompatibility complex class I-restricted CD8 SUP + cytotoxic T cells against *beta"** -*Gal"**; however, cytotoxic T-lymphocyte responses were significantly stronger after immunization with HH104 ($P < 0.05$). These novel *S"**. *typhimurium"** *attenuated"** strains constitute promising delivery systems for vaccine antigens. The qualitative differences observed in the obtained responses with different carriers may be useful for those applications in which a targeted immunomodulation is required.

17/3,AB/5 (Item 5 from file: 144)
DIALOG(R)File 144:Pascal

13808042 PASCAL No.: 98-0523313
Gene transfer in dendritic cells, induced by oral DNA vaccination with Salmonella typhimurium, results in protective immunity against a murine

fibrosarcoma
PAGLIA P; MEDINA E; ARIOLI I; GUZMAN C A; COLOMBO M P
Division of Experimental Oncology D, Istituto Nazionale per Lo Studio e la Cura dei Tumori, Milano, Italy; Division of Microbiology, GBF-National Research Centre for Biotechnology, Braunschweig, Germany
Journal: Blood, 1998, 92 (9) 3172-3176
Language: English
A live *attenuated"** AroA- auxotrophic mutant of *Salmonella"** *typhimurium"** (SL7207) has been used as carrier for the *pCMV"** beta *vector"** that contains the *beta"** -*galactosidase"** (*beta"** -*gal"**) gene under the control of the immediate early promoter of Cytomegalovirus (*CMV"**). We tested whether orally administered bacterial carrier could enter and deliver the transgene to antigen-presenting cells (APCs) through the natural enteric route of infection and whether *beta"** -*gal"** expression could generate a protective response against an aggressive murine fibrosarcoma transduced with the *beta"** -*gal"** gene (F1.A11) that behaves operationally as a tumor-associated antigen. After three courses, at 15-day intervals, mice developed both cell-mediated and systemic humoral responses to *beta"** -*gal"**. Mice vaccinated with the Salmonella harboring *pCMV"** beta , but not with *plasmid"**-less carrier, showed resistance to a challenge with F1.A11 cells. These experiments suggest that Salmonella-based DNA immunization allows us to specifically target antigen expression in vivo to APCs. To prove that the transgene is actually expressed by APCs as a function of an eukaryotic promoter, the green fluorescent protein (GFP) was placed under the control of either the eukariotic *CMV"** or a prokaryotic promoter. Using cytofluorometric analysis, GFP was detected only in splenocytes of mice receiving a Salmonella carrier harboring GFP under the *CMV"** promoter. These results indicate that transgene expression occurs because of a Salmonella-mediated gene transfer to eukaryotic cells. Finally, approximately 19% of the splenocytes expressed GFP. Among them, F4/80 SUP + macrophages and CD11c SUP b SUP r SUP i SUP g SUP h SUP t dendritic cells (DCs) were scored as positive for GFP expression. Extensive work has been performed trying to optimize the way to transfect DCs, ex vivo, with genes coding for relevant antigens. We show here, for the first time, that DCs can be directly and specifically transduced in vivo such to induce DNA vaccination against tumors.

17/3,AB/6 (Item 6 from file: 144)
DIALOG(R)File 144:Pascal

11223047 PASCAL No.: 94-0040770
Use of incompatible *plasmids"** to control expression of antigen by Salmonella typhimurium and analysis of immunogenicity in mice
ERVIN S E; SMALL P A JR; GULIG P A
Univ. Florida, coll. medicine, dep. immunology medical microbiology, Gainesville FL 32610-0266, USA
Journal: Microbial pathogenesis, 1993, 15 (2) 93-101
Language: English

17/3,AB/7 (Item 7 from file: 144)
DIALOG(R)File 144:Pascal

07601645 PASCAL No.: 87-0438922
An *attenuated"** aroA *Salmonella"** *typhimurium"** vaccine elicits humoral and cellular immunity to cloned *beta"** -*galactosidase"** in mice
BROWN A; HORMAECHE C E; DE HORMAECHE R D; WINTHER M; DOUGAN G; MASKELL B J; STOCKER B A D
Univ. Cambridge, dep. pathology, Cambridge CB2 1QP, United Kingdom
Journal: Journal of infectious Diseases, 1987, 155 (1) 86-92
Language: English

17/3,AB/8 (Item 1 from file: 266)
DIALOG(R)File 266:FEDRIP

00291534
IDENTIFYING NO.: 5R01AI33525-07 AGENCY CODE: CRISP
FLAGELLAR REGULATION AND SALMONELLA VIRULENCE
PRINCIPAL INVESTIGATOR: O'BRIEN, ALISON D
ADDRESS: UNIFORMED SERV UNIV HLTH SCI 4301 JONES BRIDGE ROAD BETHESDA, MD 20814-4799
PERFORMING ORG.: HENRY M. JACKSON FDN FOR THE ADV MIL/MED, BETHESDA, MARYLAND
SPONSORING ORG.: NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
FY : 2001
SUMMARY: A number of mutations in Salmonella typhimurium have been described that reduce the virulence of the organism in a murine typhoid model. Many of these mutations also affect survival of S. typhimurium in mouse macrophages. Among the mouse-*attenuated"** *S"**. *typhimurium"** strains that fail to persist in macrophages are strains deleted for flgA, part of the flgB operon, and the mviS locus, i.e. delta flg25. Since *plasmids"** containing the mviS region of DNA from S. typhimurium are able to restore virulence without complementing for flagella synthesis, a wild-type mviS locus appears to be necessary for the full expression of S. typhimurium pathogenicity in the mouse model. The LONG TERM GOALS of this project are to characterize the mviS locus at the molecular level and to begin to evaluate the role of mviS in the virulence of S. typhimurium. The SPECIFIC AIMS designed to achieve these objectives are: 1) to continue the molecular analysis of the mviS gene by more precisely defining the mviS region, probing other species of Salmonella for an mviS analog, and assessing whether the cloned mviS gene from typically mouse-avirulent Salmonella species enhances the virulence of mviS S. typhimurium; 2) to construct isogenic strains of S. typhimurium that differ only at the mviS locus and to compare the virulence of the flg+mviS+ and flg+mviS- strains for C57BL/6J mice and the survival of each member of the isogenic pair: i) in macrophages from innately salmonella-resistant and salmonella-susceptible macrophages, ii) at low pH, and iii) in the presence of the defensin-like compound protamine; 3) to determine whether mviS functions to regulate expression of other S. typhimurium genes and characterize any mviS-regulated gene(s); 4) to assess the expression and regulation of mviS by generating a fusion of the mviS promoter to the reporter gene *beta"**-*galactosidase"** and monitoring the level of *beta"**-*galactosidase"** : i) under a variety of in vitro growth conditions, ii) inside infected epithelial cells and macrophages, and iii) in tissue homogenates from infected mice. Lastly, the cell type(s) in which the mviS-fusion product is expressed will be determined by histochemical analysis of tissue from infected mice.

17/3,AB/9 (Item 1 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

09035362 GENUINE ARTICLE#: YL439 NUMBER OF REFERENCES: 50
TITLE: Oral somatic transgene vaccination using *attenuated"** *S"**-
*typhimurium"**
AUTHOR(S): Darji A (REPRINT); Guzman CA; Gerstel B; Wachholz P; Timmis KN;
Wehland J; Chakraborty T; Weiss S
CORPORATE SOURCE: GESELL BIOTECHNOL FORSCH MBH,NATL RES CTR BIOTECHNOL, DIV
CELL BIOL & IMMUNOL, MASCHERODER WEG 1/D-38124 BRAUNSCHWEIG//GERMANY/
(REPRINT); GESELL BIOTECHNOL FORSCH MBH,NATL RES CTR BIOTECHNOL, DIV
MICROBIOL/D-38124 BRAUNSCHWEIG//GERMANY/; UNIV GIESSEN,INST MED
MICROBIOL/D-35392 GIESSEN//GERMANY/
PUBLICATION TYPE: JOURNAL
PUBLICATION: CELL, 1997, V91, N6 (DEC 12), P765-775
PUBLISHER: CELL PRESS, 1050 MASSACHUSETTES AVE, CIRCULATION DEPT,
CAMBRIDGE, MA 02138
ISSN: 0092-8674
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: An *attenuated"** strain of *S"**. *typhimurium"** has been used
as a vehicle for oral genetic immunization. Eukaryotic expression
*vectors"** containing truncated genes of *ActA"** and
*listeriolysin"**-two virulence factors of Listeria monocytogenes-have
been used to transform S. typhimurium aroA. Multiple or even single
oral immunizations with such transformants induced excellent cellular
and humoral responses. In addition, protective immunity was induced
with *listeriolysin"** transformants. The quality of the responses
suggested a transfer of *plasmid"** DNA from the bacterial carrier to
the host. Such transfer was unequivocally shown in vitro with primary
peritoneal macrophages. We describe a highly versatile system for
antigen delivery, identification of protective antigens for
vaccination, and efficient generation of antibodies against the product
of open reading frames present on virtually any DNA segment.

17/3,AB/10 (Item 2 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

08141360 GENUINE ARTICLE#: WE900 NUMBER OF REFERENCES: 61
TITLE: Simultaneous prevention of glutamine synthesis and high-affinity
transport *attenuates"** *Salmonella"** *typhimurium"** virulence
AUTHOR(S): Klose KE; Mekalanos JJ (REPRINT)
CORPORATE SOURCE: HARVARD UNIV,SCH MED, DEPT MICROBIOL & MOL GENET, 200
LONGWOOD AVE/BOSTON//MA/02115 (REPRINT); HARVARD UNIV,SCH MED, DEPT
MICROBIOL & MOL GENET/BOSTON//MA/02115; HARVARD UNIV,SCH MED, SHIPLEY
INST MED/BOSTON//MA/02115
PUBLICATION TYPE: JOURNAL
PUBLICATION: INFECTION AND IMMUNITY, 1997, V65, N2 (FEB), P587-596
PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW,
WASHINGTON, DC 20005-4171
ISSN: 0019-9567
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: In Salmonella typhimurium, transcription of the glnA gene
(encoding glutamine synthetase) is under the control of the

nitrogen-regulatory (ntr) system comprising the alternate sigma factor sigma(54) (NtrA) and the two-component sensor-transcriptional activator pair NtrB and NtrC. The glnA, ntrB, and ntrC genes form an operon, We measured the virulence of S. typhimurium strains with nitrogen-regulatory mutations after intraperitoneal (i.p.) or oral inoculations of BALB/c mice, Strains with single mutations in glnA, ntrA, ntrB, or ntrC had i.p. 50% lethal doses (LD(50)s) of <10 bacteria, similar to the wild-type strain, However, a strain with a Delta(glnA-ntrC) operon deletion had an i.p. LD(50) of >10(5) bacteria, as did Delta glnA ntrA and Delta glnA ntrC strains, suggesting that glnA strains require an ntr-transcribed gene for full virulence, High-level transcription of the glutamine transport operon (glnHPQ) is dependent upon both ntrA and ntrC, as determined by glnHp-*lacZ"** fusion measurements. Moreover, Delta glnA glnH and Delta glnA glnQ strains are attenuated, similar to Delta glnA ntrA and Delta glnA ntrC strains, These results reveal that access of S. typhimurium to host glutamine depends on the ntr system, which apparently is required for the transcription of the glutamine transport genes, The Delta(glnA-ntrC) strain exhibited a reduced ability to survive within the macrophage cell line J774, identifying a potential host environment with low levels of glutamine, Finally, the Delta(glnA-ntrC) strain, when inoculated at doses as low as 10 organisms, provided mice with protective immunity against challenge by the wild-type strain, demonstrating its potential use as a live vaccine.

17/3,AB/11 (Item 3 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

07977870 GENUINE ARTICLE#: VV676 NUMBER OF REFERENCES: 50
TITLE: Development of antigen-delivery systems, based on the Escherichia coli hemolysin secretion pathway
AUTHOR(S): Gentschev I; Mollenkopf H; Sokolovic Z; Hess J; Kaufmann SHE; Goebel W (REPRINT)
CORPORATE SOURCE: THEODOR BOVERI INST BIOWISSENSCH,LEHRSTUHL MIKROBIOL, HUBLAND/D-97074 WURZBURG//GERMANY/ (REPRINT); THEODOR BOVERI INST BIOWISSENSCH,LEHRSTUHL MIKROBIOL/D-97074 WURZBURG//GERMANY/; UMEA UNIV,DEPT IMMUNOL/D-89070 ULM//GERMANY/
PUBLICATION TYPE: JOURNAL
PUBLICATION: GENE, 1996, V179, N1 (NOV 7), P133-140
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS
ISSN: 0378-1119
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: We describe the development of *plasmid"** *vectors"** carrying the expression sites, an hlyA cassette and the secretion genes of Escherichia coli hemolysin. These allow the synthesis and secretion of heterologous microbial antigens in E. coli and attenuated Salmonella aroA strains. Genes or gene fragments encoding microbial antigens are inserted in-frame into a residual part of the hlyA gene which essentially encodes the HlyA secretion signal (HlyA(s)). In general, the fused genes, carrying the hlyA(s) sequence at the 3' terminus, are efficiently expressed, and the synthesized antigens are secreted into the culture supernatant of the producing strain. Attenuated Salmonella strains synthesizing either HlyA(s)-fused *listeriolysin"** or p60 of Listeria monocytogenes were constructed by this procedure and shown to provide protective immunity against L. monocytogenes in mice. The most effective protection was obtained when these microbial antigens were

secreted by the attenuated Salmonella strains. We further present new approaches which may allow the application of this antigen-delivery system to any microbial antigen.

17/3,AB/12 (Item 4 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

07414825 GENUINE ARTICLE#: UM766 NUMBER OF REFERENCES: 32
TITLE: AN ESCHERICHIA COLI HEMOLYSIN TRANSPORT SYSTEM-BASED *VECTOR"** FOR THE EXPORT OF POLYPEPTIDES - EXPORT OF SHIGA-LIKE TOXIN IIEB SUBUNIT BY SALMONELLA TYPHIMURIUM AROA
AUTHOR(S): TZSCHASCHEL BD; GUZMAN CA (Reprint); TIMMIS KN; DELORENZO V
CORPORATE SOURCE: NATL RES CTR BIOTECHNOL,DIV MICROBIOL,MASCHERODER WEG 1/D-38124 BRAUNSCHWEIG//GERMANY/ (Reprint); NATL RES CTR BIOTECHNOL,DIV MICROBIOL/D-38124 BRAUNSCHWEIG//GERMANY/; CSIC,CTR INVEST BIOL/E-28006 MADRID//SPAIN/
PUBLICATION: NATURE BIOTECHNOLOGY, 1996, V14, N6 (JUN), P765-769
ISSN: 1087-0156
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE
ABSTRACT: The export of Escherichia coli hemolysin across the cytoplasmic and the outer membranes requires the COOH-terminal signal sequence of HlyA, the two specific translocator proteins HlyB and HlyD, and the outer membrane protein TolC. We have developed an export cloning system that is composed of two *vectors"**: one in which the fusion of the desired gene with the 3'-end of hlyA is generated, and a second in which the sequences containing the fusion are combined with the accessory genes hlyB and hlyD, thereby reconstructing the natural organization of the *hly"** locus. in the second *vector"** the fusion and the accessory genes are flanked by NotI sites, allowing subcloning of the whole cluster into a variety of minitransposons to achieve the stable integration of the constructs into the chromosome of Gram-negative bacteria. Since some applications may require the production of transcriptional fusions, an alternative version of the system provides the efficient translation initiation region of T7 phage gene 10 upstream of the fusion protein coding sequence. The usefulness of the system was assessed by constructing a fusion between the gene encoding the B subunit of Shiga-like toxin lie and the 3'-end of hlyA. An *attenuated"** *Salmonella"** *typhimurium"** vaccine strain harboring the resulting construct, either in multicopy or monocopy, efficiently expressed and exported the chimeric protein. We anticipate that this system will lead to a higher stability of the engineered function and permit a faithful monitoring of the export of the recombinant peptide under physiologic single-copy conditions.

17/3,AB/13 (Item 1 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

01388767
Vaccination against host cell-associated herpesviruses
Impfung gegen Wirtzelle-assoziierte Herpesviren
Vaccination contre des virus herpes associes aux cellules-hotes
PATENT ASSIGNEE:
Lohmann Animal Health GmbH & Co. KG, (2189640), Heinz-Lohmann-Strasse 4, 27472 Cuxhaven, (DE), (Applicant designated States: all)

INVENTOR:
Fehler, Frank, c/o Lohmann Animal Health GmbH & Co, Heinz-Lohmann-Strasse 4, 27454 Cuxhaven, (DE)
Osterrieder, Klaus, c/o Inst. of Molecular & Cel., Boddenblick 5a, 17498 Insel Riems, (DE)
LEGAL REPRESENTATIVE:
Prins, Adrianus Willem et al (20903), Vereenigde, Nieuwe Parklaan 97, 2587 BN Den Haag, (NL)
PATENT (CC, No, Kind, Date): EP 1178111 A1 020206 (Basic)
APPLICATION (CC, No, Date): EP 2000202757 000803;
DESIGNATED STATES: AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE
EXTENDED DESIGNATED STATES: AL; LT; LV; MK; RO; SI
INTERNATIONAL PATENT CLASS: C12N-015/38; C12N-001/21; A61K-031/70; A61K-039/112; C12N-015/10

ABSTRACT EP 1178111 A1
The invention relates to the field of so-called host cell-associated herpesviruses such as Marek's disease like virus (MDV) of poultry and of Varicella Zoster Virus (VZV) of man and to vaccination against disease caused by these viruses. The invention provides a vaccine directed against an infection caused by an herpesvirus that is essentially host cell associated comprising a recombinant viral genome derived from said herpesvirus, said genome allowing recombination essentially free of said host cell.
ABSTRACT WORD COUNT: 76
NOTE:
Figure number on first page: NONE

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	200206	551
SPEC A	(English)	200206	9769
Total word count - document A			10320
Total word count - document B			0
Total word count - documents A + B			10320

17/3,AB/14 (Item 2 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

01258934
Oral recombinant lactobacilli vaccines
Oraler Impfstoff enthaltend rekombinanten Lactobacilli
Vaccin oral contenant des Lactobacilli recombines
PATENT ASSIGNEE:
NEDERLANDSE ORGANISATIE VOOR TOEGEPAST-NATUURWETENSCHAPPELIJK ONDERZOEK TNO, (285526), Schoemakerstraat 97, P.O. Box 60680, 2628 VK Delft, (NL), (Applicant designated States: all)
INVENTOR:
Shaw, David, Michael, 18 Austins-Mead, HP3 OJX Bovingdon, Hertfordshire, (GB)
Leer, Robert Jan, Kompas 7, 3904 PN Veenendaal, (NL)
Pouwels, Peter, Delftweg 14, 2289 AJ Rijswijk, (NL)
LEGAL REPRESENTATIVE:
Wright, Simon Mark et al (72652), J.A. Kemp & Co. 14 South Square Gray's

Inn, London WC1R 5LX, (GB)
PATENT (CC, No, Kind, Date): EP 1084709 A1 010321 (Basic)
EP 1084709 A9 010516
APPLICATION (CC, No, Date): EP 99203056 990917;
DESIGNATED STATES: AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE
EXTENDED DESIGNATED STATES: AL; LT; LV; MK; RO; SI
INTERNATIONAL PATENT CLASS: A61K-039/00; C12N-015/74

ABSTRACT EP 1084709 A1
The present invention relates to an oral vaccine comprising recombinant lactic acid bacteria expressing heterologous antigen in vivo intracellularly and/or the surface of the lactic acid bacterium as specific immunogenicity eliciting component for eliciting immunogenicity against the heterologous antigen, characterised in that the recombinant lactic acid bacterium is a Lactobacillus plantarum.
Preferably, the recombinant Lactobacillus plantarum comprises an expression *vector"** capable of expressing the heterologous antigen intracellularly and/or such that the heterologous antigen is exposed on the cell surface under conditions present in the gastrointestinal tract.
The recombinant Lactobacillus plantarum is preferably a recombinant Lactobacillus plantarum 256.
The invention also relates to a recombinant Lactobacillus plantarum, more specifically a recombinant strain of Lactobacillus plantarum 256, for use in the vaccines of the invention; as well as to an expression *vector"** suitable for intracellular expression or exposure of a heterologous antigen encoded thereon, said expression *vector"** providing expression in a Lactobacillus plantarum of the heterologous antigen under conditions existing in the gastrointestinal tract.
ABSTRACT WORD COUNT: 163
NOTE:
Figure number on first page: NONE

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	200112	808
SPEC A	(English)	200112	10117
Total word count - document A			10925
Total word count - document B			0
Total word count - documents A + B			10925

17/3,AB/15 (Item 3 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

01071702
Live attenuated salmonella vaccine
Lebender attenuierter Salmonella Impfstoff
Vaccin a base de Salmonella vivant attenue
PATENT ASSIGNEE:
VRIJE UNIVERSITEIT BRUSSEL, (2116290), Pleinlaan 2, 1050 Brussel, (BE), (Applicant designated States: all)
INVENTOR:
Gubbels, Elina Frieda Irena Albert, Eikblokstraatt 13, 2100 Antwerpen-Deurne, (BE)
De Greve, Henri Marcel Jozef, Jef Lambeauxlaan 11, 1060 Brussel, (BE)

Hernalsteens, Jean-Pierre Ernest Clement, Francois Desmedtstraat 156, 1150 Sint-Pieters-Woluwe, (BE)
LEGAL REPRESENTATIVE:
Van Malderen, Joelle et al (75971), Office Van Malderen, Place Reine Fabiola 6/1, 1083 Bruxelles, (BE)
PATENT (CC, No, Kind, Date): EP 943681 A1 990922 (Basic)
APPLICATION (CC, No, Date): EP 98870019 980122;
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE
EXTENDED DESIGNATED STATES: AL; LT; LV; MK; RO; SI
INTERNATIONAL PATENT CLASS: C12N-015/11; A61K-039/112; C07K-014/255

ABSTRACT EP 943681 A1
The present invention is related to a vaccine for inducing an immune response to a Salmonella strain in an animal, including a human, characterised in that it comprises a pharmaceutically acceptable carrier and a genetically modified Salmonella strain which is in an amount effective to produce an immune response in said animal, including human, and comprises a modification in its wild type DNA sequence SEQ ID NO 01, any of the DNA sequences from the same operon as SEQ ID NO 01 and/or any regulatory sequence of any of the said DNA sequences.
ABSTRACT WORD COUNT: 94
NOTE:
Figure number on first page: 2

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	9938	852
SPEC A	(English)	9938	6764
Total word count - document A			7616
Total word count - document B			0
Total word count - documents A + B			7616

17/3,AB/16 (Item 4 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

00981501
A micro-organism having reduced adaption to a particular environment
Ein Mikroorganism mit einer reduzierten Anpassung einer bestimmten Umgebung
Micro-organisme ayant une adaption reduite pour un environnement particulier
PATENT ASSIGNEE:
IMPERIAL COLLEGE INNOVATIONS LIMITED, (2530151), Sherfield Building, Imperial College, London SW7 2AZ, (GB), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;GR;IE;IT;LI;LU;MC;NL;PT;SE)
INVENTOR:
Holden, David William, Dept of Inf. Dis.,Imperial, College of Sc.,Techn.and Med.,Flowers Build., Armstrong Rd., London SW7 2AZ, (GB)
Shea Jacqueline Elizabeth, Microscience Ltd, 545 Eskdale Rd.,Winnersh Triangle, Wokingham, Berkshire RG41 5TU, (GB)
Hensel Michael,The Inst.for Clinic.Microb.,Immuno., and Hygiene, University Erlangen,Wasserturmstr.3/5, 91054 Erlangen, (DE)
LEGAL REPRESENTATIVE:
Bassett, Richard Simon (52833), Eric Potter Clarkson, Park View House, 58 The Ropewalk, Nottingham NG1 5DD, (GB)

PATENT (CC, No, Kind, Date): EP 889120 A1 990107 (Basic)
APPLICATION (CC, No, Date): EP 98201907 951211;
PRIORITY (CC, No, Date): GB 9424921 941209; GB 9501881 950131; GB 9509239 950505
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE
RELATED PARENT NUMBER(S) - PN (AN):
EP 796341 (EP 95939362)
RELATED DIVISIONAL NUMBER(S) - PN (AN):
(EP 2001205191)
INTERNATIONAL PATENT CLASS: C12N-001/21; C12N-015/31; A61K-035/74; C07K-014/255; C12N-015/11; C12Q-001/68; A61K-039/112;

ABSTRACT EP 889120 A1
A Microorganism having a reduced adaptation to a particular environment identified with a method comprising the steps of: (1) providing a plurality of microorganisms each of which is independently mutated by the insertional inactivation of a gene with a nucleic acid comprising a unique marker sequence so that each mutant contains a different marker sequence, or clones of the said microorganism; (2) providing individually a stored sample of each mutant produced by step (1) and providing individually stored nucleic acid comprising the unique marker sequence from each individual mutant; (3) introducing a plurality of mutants produced by step (1) into the said particular environment and allowing those microorganisms which are able to do so to grow in the said environment; (4) retrieving microorganisms from the said environment or a selected part thereof and isolating the nucleic acid from retrieved microorganisms; (5) comparing any marker sequences in the nucleic acid isolated in step (4) to the unique marker sequence of each individual mutant stored as in step (2); and (6) selecting an individual mutant which does not contain any of the marker sequences as isolated in step (4). Furthermore, the invention relates to: a gene isolated from the microorganism, a vaccine comprising the microorganism, a polypeptide encoded by the gene, a method for identifying compounds interfering with the function of the gene e.g. by antisense. The microorganism is exemplified with Salmonella typhimurium and its VGC2 gene.
ABSTRACT WORD COUNT: 236

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	9901	972
SPEC A	(English)	9901	21340
Total word count - document A			22312
Total word count - document B			0
Total word count - documents A + B			22312

17/3,AB/17 (Item 5 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

00784440
IDENTIFICATION OF GENES WHICH ALLOW A MICROORGANISM TO ADAPT TO A PARTICULAR ENVIRONMENT
IDENTIFIZIERUNG VON GENEN VERANTWORTLICH FUR DIE ANPASSUNG VON MIKROORGANISMEN AN EINE BESTIMMTE UMGEBUNG
IDENTIFICATION DE GENES RESPONSABLES POUR L'ADAPTATION DE MICROORGANISMES A

UN ENVIRONNEMENT PARTICULIER.
PATENT ASSIGNEE:
IMPERIAL COLLEGE INNOVATIONS LIMITED, (2530150), 47 Prince's Gate, London SW7 2QA, (GB), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;GR;IE;IT;LI;LU;MC;NL;PT;SE)
INVENTOR:
HOLDEN, David, William Dept. of Infect. Diseases, and Bacter. Royal Postgraduate Med. School, Hammersmith Hospital Du Cane Road London, (GB)
LEGAL REPRESENTATIVE:
Bassett, Richard Simon et al (52833), Eric Potter Clarkson, Park View House, 58 The Ropewalk, Nottingham NG1 5DD, (GB)
PATENT (CC, No, Kind, Date): EP 796341 A2 970924 (Basic)
EP 796341 B1 980923
WO 9617951 960613
APPLICATION (CC, No, Date): EP 95939362 951211; WO 95GB2875 951211
PRIORITY (CC, No, Date): GB 9424921 941209; GB 9501881 950131; GB 9509239 950505
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE
INTERNATIONAL PATENT CLASS: C12Q-001/04; C12Q-001/68; C12N-015/10;
NOTE:
No A-document published by EPO
LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS B	(English)	9839	1009
CLAIMS B	(German)	9839	1003
CLAIMS B	(French)	9839	1111
SPEC B	(English)	9839	21206
Total word count - document A			0
Total word count - document B			24329
Total word count - documents A + B			24329

17/3,AB/18 (Item 6 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

00724155
ANTIGENS PROTECTIVE AGAINST (ECHINOCOCCUS GRANULOSUS) INFECTION AND VACCINES CONTAINING SUCH ANTIGENS
SCHUTZENDE ANTIGENE GEGEN EINE (ECHINOCOCCUS GRANULOSUS) INFEKTION UND IMPFSTOFFE DIE ENTSPRECHENDE ANTIGENE ENTHALTEN
ANTIGENES PROTECTEURS CONTRE L'INFECTION PAR ECHINOCOCCUS GRANULOSUS ET VACCINS CONTENANT DE TELS ANTIGENES
PATENT ASSIGNEE:
NEW ZEALAND PASTORAL AGRICULTURE RESEARCH INSTITUTE LIMITED, (1694770), Peat Marwick Tower, 85 Alexandra Street, Hamilton, (NZ), (Proprietor designated states: all)
THE UNIVERSITY OF MELBOURNE, (202599), Grattan Street, Parkville, Victoria 3052, (AU), (Proprietor designated states: all)
INVENTOR:
HEATH, David Duncan, 76 Paremata Road, Ivey Bay, Paremata, (NZ)
LAWRENCE, Stephen, Bruce, 94 Plateau Road, Upper Hutt, (NZ)
RALSTON, Mark, John, 106 Avro Road, RD 1, Upper Hutt, (NZ)
MAASS, David Richard, 5 Latham Road, York Bay, Wellington, (NZ)
LIGHTOWLERS, Marshall, William, 176 Osbourne Street, Williamstown, VIC,

(AU)
LEGAL REPRESENTATIVE:
Armitage, Ian Michael et al (27761), MEWBURN ELLIS York House 23 Kingsway , London WC2B 6HP, (GB)
PATENT (CC, No, Kind, Date): EP 629131 A1 951129 (Basic)
EP 629131 B1 010627
WO 9316722 930902
APPLICATION (CC, No, Date): EP 93905659 930222; WO 93NZ7 930222
PRIORITY (CC, No, Date): NZ 24168892 920221
DESIGNATED STATES: DE; ES; FR; GB; IT; PT
INTERNATIONAL PATENT CLASS: C12N-015/12; C12N-001/21; C12N-015/70; C12N-015/79; C12N-007/01; C07K-014/435; A61K-039/00; A61K-039/395; C12Q-001/68
NOTE:
No A-document published by EPO
LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS B	(English)	200126	924
CLAIMS B	(German)	200126	870
CLAIMS B	(French)	200126	956
SPEC B	(English)	200126	9790
Total word count - document A			0
Total word count - document B			12540
Total word count - documents A + B			12540

17/3,AB/19 (Item 7 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

00650997
HYBRID DNA THAT CODES FOR A SUBUNIT OF A BACTERIAL TOXIN WITH A HEMOLYSIN
HYBRID-DNA, DIE EINE UNTEREINHEIT EINES BAKTERIELLEN TOXINS MIT EINER HAEMOLYSIN KODIERT
ADN HYBRIDE QUI CODE UNE UNITE SECONDAIRE D'UNE TOXINE BACTERIENNE AVEC UNE HEMOLYSINE
PATENT ASSIGNEE:
Gesellschaft fur Biotechnologische Forschung mbH (GBF), (235880), Mascheroder Weg 1, D-38124 Braunschweig, (DE), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;IT;LI;NL;SE)
INVENTOR:
BRAHMBHATT, Himanshu, Mascheroder Weg 1, D-3300 Braunschweig, (DE)
SU, Guo-fu, Mascheroder Weg 1, D-3300 Braunschweig, (DE)
WEHLAND, Jurgen, Mascheroder Weg 1, D-3300 Braunschweig, (DE)
TIMMIS, Kenneth, N., Mascheroder Weg 1, D-3300 Braunschweig, (DE)
LEGAL REPRESENTATIVE:
Boeters, Hans Dietrich, Dr. et al (2193), Patentanwalte Boeters & Bauer, Bereiteranger 15, 81541 Munchen, (DE)
PATENT (CC, No, Kind, Date): EP 627006 A1 941207 (Basic)
EP 627006 B1 970502
WO 9316186 930819
APPLICATION (CC, No, Date): EP 93905242 930212; WO 93EP348 930212
PRIORITY (CC, No, Date): DE 4204737 920217; DE 4219696 920616
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; IT; LI; NL; SE
INTERNATIONAL PATENT CLASS: C12N-015/62; C12N-015/74; C07K-007/00; C07K-014/00;
NOTE:

No A-document published by EPO
LANGUAGE (Publication,Procedural,Application): German; German; German
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS B	(English)	EPAB97	232
CLAIMS B	(German)	EPAB97	210
CLAIMS B	(French)	EPAB97	261
SPEC B	(German)	EPAB97	4301
Total word count - document A			0
Total word count - document B			5004
Total word count - documents A + B			5004

17/3,AB/20 (Item 8 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

00557240
EXPRESSION OF RECOMBINANT PROTEINS IN ATTENUATED BACTERIA
EXPRESSION REKOMBINANTER PROTEINE IN ATTENUIERTEN BAKTERIEN
EXPRESSION DE PROTEINES DE RECOMBINAISON DANS DES BACTERIES ATTENUEES
PATENT ASSIGNEE:
THE WELLCOME FOUNDATION LIMITED, (201576), Glaxo Wellcome House, Berkeley Avenue, Greenford, Middlesex UB6 0NN, (GB), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;GR;IT;LI;LU;MC;NL;SE)
INVENTOR:
CHARLES, Ian George, Langley Court, Beckenham, Kent BR3 3BS, (GB)
CHATFIELD, Steven Neville, Langley Court, Beckenham, Kent BR3 3BS, (GB)
FAIRWEATHER, Neil Fraser, Langley Court, Beckenham, Kent BR3 3BS, (GB)
LEGAL REPRESENTATIVE:
Woods, Geoffrey Corlett et al (48721), J.A. KEMP & CO. 14 South Square Gray's Inn, London WC1R 5LX, (GB)
PATENT (CC, No, Kind, Date): EP 574466 A1 931222 (Basic)
EP 574466 B1 990519
WO 9215689 920917
APPLICATION (CC, No, Date): EP 92905914 920305; WO 92GB387 920305
PRIORITY (CC, No, Date): GB 9104596 910305; GB 9121208 911004
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LI; LU; MC; NL; SE
INTERNATIONAL PATENT CLASS: C12N-015/74; C12N-015/70; C12N-015/31; A61K-039/112; A61K-039/10; C12N-001/21; C12N-001/21; C12R-001/42
NOTE:
No A-document published by EPO
LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS B	(English)	9920	999
CLAIMS B	(German)	9920	918
CLAIMS B	(French)	9920	1090
SPEC B	(English)	9920	3690
Total word count - document A			0
Total word count - document B			6697
Total word count - documents A + B			6697

17/3,AB/21 (Item 9 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

00556330
IMPROVED VACCINES
VERBESSERTE IMPFSTOFFE
VACCINS AMELIORES
PATENT ASSIGNEE:
THE GENERAL HOSPITAL CORPORATION, (370407), Office of Technology Affairs, Thirteenth Street, Building 149, Suite 1101, Charlestown, MA 02129, (US), (Proprietor designated states: all)
THE PRESIDENT AND FELLOWS OF HARVARD COLLEGE, (227952), 17 Quincy Street, Cambridge, MA 02114, (US), (Proprietor designated states: all)
INVENTOR:
MILLER, Samuel, I., III, 144 Jordan Road, Brookline, MA 02146, (US)
MEKALANOS, John, J., One Walker Court, Cambridge, MA 02138, (US)
LEGAL REPRESENTATIVE:
Rackham, Anthony Charles et al (35051), Lloyd Wise, Tregear & Co., Commonwealth House, 1-19 New Oxford Street, London WC1A 1LW, (GB)
PATENT (CC, No, Kind, Date): EP 563311 A1 931006 (Basic)
EP 563311 A1 950809
EP 563311 B1 000315
WO 9211361 920709
APPLICATION (CC, No, Date): EP 92904089 911218; WO 91US9604 911218
PRIORITY (CC, No, Date): US 629602 901218
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LI; LU; MC; NL; SE
INTERNATIONAL PATENT CLASS: C12N-015/74; A61K-039/112; A61K-039/40; C12Q-001/10; C12Q-001/68
NOTE:
No A-document published by EPO
LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS B	(English)	200011	1048
CLAIMS B	(German)	200011	1043
CLAIMS B	(French)	200011	1130
SPEC B	(English)	200011	10556
Total word count - document A			0
Total word count - document B			13777
Total word count - documents A + B			13777

17/3,AB/22 (Item 10 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

00554538
RECOMBINANT AVIRULENT SALMONELLA ANTIFERTILITY VACCINES
REKOMBINANTE AVIRULENTE SALMONELLA ANTIFRUCHTBARKEITSIMPFSTOFFE
VACCINS ANTICONCEPTIONNELS DE SALMONELLA AVIRULENT RECOMBINANT
PATENT ASSIGNEE:
WASHINGTON UNIVERSITY, (645441), Campus Box 1137, 1 Brookings Drive, St. Louis, Missouri 63130-4899, (US), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;GR;IT;LI;LU;NL;SE)
INVENTOR:
CURTISS, Roy, III, 6065 Lindell Boulevard, St. Louis, MO 63117, (US)
TUNG, Kenneth, S., K., 9 Old Farm Road, Charlottsville, VA 22901, (US)
LEGAL REPRESENTATIVE:
Kolb, Helga, Dr. Dipl.-Chem. et al (49371), Hoffmann Eitle, Patent- und

Rechtsanwalte, Postfach 81 04 20, 81904 Munchen, (DE)
PATENT (CC, No, Kind, Date): EP 558631 A1 930908 (Basic)
EP 558631 A1 951122
EP 558631 B1 990317
WO 9209684 920611
APPLICATION (CC, No, Date): EP 92901072 911120; WO 91US8688 911120
PRIORITY (CC, No, Date): US 615720 901121; US 791347 911118
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LI; LU; NL; SE
INTERNATIONAL PATENT CLASS: C12N-015/53; C12N-001/21; A61K-039/02; A61K-039/108; A61K-039/112; C12N-009/02; C12N-015/01; C12N-009/04;
NOTE:
No A-document published by EPO
LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS B	(English)	9911	948
CLAIMS B	(German)	9911	936
CLAIMS B	(French)	9911	1079
SPEC B	(English)	9911	20977
Total word count - document A			0
Total word count - document B			23940
Total word count - documents A + B			23940

17/3,AB/23 (Item 11 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

00535203
Treponema hyodysenteriae vaccine.
Impfstoff gegen Trepanoma hyodysenteriae.
Vaccin contre le Trepanoma hyodysenteriae.
PATENT ASSIGNEE:
DUPHAR INTERNATIONAL RESEARCH B.V, (216651), C.J. van Houtenlaan 36, NL-1380 AC Weesp, (NL), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;GR;IE;IT;LI;LU;NL;PT;SE)
INVENTOR:
Muir, Susie Jane c/o Octrooibureau Zoan b.v., P.O. Box 140, NL-1380 AC Weesp, (NL)
Koopmans, Marcel B.H. c/o Octrooibureau Zoan b.v., P.O. Box 140, NL-1380 AC Weesp, (NL)
Kusters, Johannes G. c/o Octrooibureau Zoan b.v., P.O. Box 140, NL-1380 AC Weesp, (NL)
LEGAL REPRESENTATIVE:
Wileman, David Francis, Dr. et al (46002), c/o Wyeth Laboratories Huntercombe Lane South, Taplow Maidenhead Berkshire SL6 OPH, (GB)
PATENT (CC, No, Kind, Date): EP 551671 A1 930721 (Basic)
APPLICATION (CC, No, Date): EP 92203781 921021;
PRIORITY (CC, No, Date): EP 91202766 911025; EP 92202274 920724
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IE; IT; LI; LU; NL; PT; SE
INTERNATIONAL PATENT CLASS: C12N-015/31; C12P-021/00; A61K-039/02; A61K-039/40;

ABSTRACT EP 551671 A1
The present invention is concerned with vaccine for combating Treponema hyodysenteriae infection in swine containing proteins or polypeptides typical of the hemolysin protein of Treponema hyodysenteriae or

containing recombinant polynucleotides having as part thereof a polynucleotide coding for said protein or polypeptide, and also is concerned with the preparation of said proteins, polypeptides and polynucleotides.
ABSTRACT WORD COUNT: 57

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	EPABF1	199
SPEC A	(English)	EPABF1	6817
Total word count - document A			7016
Total word count - document B			0
Total word count - documents A + B			7016

17/3,AB/24 (Item 12 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

00534494
Treponema hyodysenteriae vaccine.
Treponema-Hyodysenteriae Vakzin.
Vaccin de treponema hyodysenteriae.
PATENT ASSIGNEE:
DUPHAR INTERNATIONAL RESEARCH B.V, (216651), C.J. van Houtenlaan 36, NL-1380 AC Weesp, (NL), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;GR;IE;IT;LI;LU;NL;PT;SE)
INVENTOR:
Koopman, Marcel B.H., c/o Octrooibureau Zoan b.v., P.O. Box 140, NL-1380 AC Weesp, (NL)
Kusters, Johannes G., c/o Octrooibureau Zoan b.v., P.O. Box 140, NL-1380 AC Weesp, (NL)
LEGAL REPRESENTATIVE:
Breepoel, Peter M. (60271), Octrooibureau Zoan B.V. P.O. Box 140, NL-1380 AC Weesp, (NL)
PATENT (CC, No, Kind, Date): EP 534526 A1 930331 (Basic)
APPLICATION (CC, No, Date): EP 92202796 920914;
PRIORITY (CC, No, Date): EP 91202478 910925; EP 92202273 920724
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IE; IT; LI; LU; NL; PT; SE
INTERNATIONAL PATENT CLASS: C12N-015/31; A61K-039/02; C07K-013/00;

ABSTRACT EP 534526 A1
The present invention is concerned with vaccine for combating Treponema hyodysenteriae infection in swine containing proteins or polypeptides typical of the endoflagellum sheath protein of Treponema hyodysenteriae or containing recombinant polynucleotides having as part thereof a polynucleotide coding for said protein or polypeptide, and also is concerned with the preparation of said proteins, polypeptides and polynucleotides.
ABSTRACT WORD COUNT: 58

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	EPABF1	206
SPEC A	(English)	EPABF1	9249

Total word count - document A 9455
Total word count - document B 0
Total word count - documents A + B 9455

17/3,AB/25 (Item 13 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

00517596
Recombinant live vaccines against Gram-negative enteric pathogens
Rekombinanter lebender Impfstoff gegen Gram-negative enterische Pathogene
Vaccin recombinant vivant contre des agents pathogene enteriques Gram-negatifs
PATENT ASSIGNEE:
SCHWEIZERISCHES SERUM- & IMPFINSTITUT BERN, (1194260), Postfach 2707, CH-3001 Bern, (CH), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;GR;IT;LI;LU;MC;NL;PT;SE)
INVENTOR:
Viret, Jean-Francois, Gartenstrasse 50, CH-3177 Laupen, (CH)
Cryz, Stanly J., Jr., Hosstudenweg 11, CH-3176 Neuenegg, (CH)
Favre, Didier, Pfrunstrasse 28, CH-3176 Neuenegg, (CH)
LEGAL REPRESENTATIVE:
VOSSIUS & PARTNER (100311), Postfach 86 07 67, 81634 Munchen, (DE)
PATENT (CC, No, Kind, Date): EP 564689 A1 931013 (Basic)
EP 564689 B1 970723
APPLICATION (CC, No, Date): EP 92106281 920410;
PRIORITY (CC, No, Date): EP 92106281 920410
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LI; LU; MC; NL; PT; SE
INTERNATIONAL PATENT CLASS: A61K-039/112; A61K-039/116;

ABSTRACT EP 564689 A1
The present invention relates to recombinant live attenuated bacterial strains expressing O-serotype determinants of gram-negative enteric pathogens in a form covalently bound to a lipopolysaccharide (LPS) core, wherein the resulting LPS molecules are essentially indistinguishable from the ones present in the original pathogenic strains. Such recombinant carrier strains may be used as live vaccines for oral immunization against e.g. bacillary dysentery caused by virulent strains of Shigella spp.
ABSTRACT WORD COUNT: 70

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	EPABF1	575
CLAIMS B	(English)	9707W4	628
CLAIMS B	(German)	9707W4	576
CLAIMS B	(French)	9707W4	670
SPEC A	(English)	EPABF1	12786
SPEC B	(English)	9707W4	12656

Total word count - document A 13362
Total word count - document B 14530
Total word count - documents A + B 27892

17/3,AB/26 (Item 14 from file: 348)

00517216
Stable pura *vectors"** and uses thereof
Stabile pura-Vektoren und ihre Verwendung
Vecteurs pura stables et leur utilisation

PATENT ASSIGNEE:
AMERICAN CYANAMID COMPANY, (212594), One Cyanamid Plaza, Wayne, NJ 07470-8426, (US), (Proprietor designated states: all)
INVENTOR:
Brey III, Robert Newton, 74 Sagamore Drive, Rochester, New York 14617, (US)
Fulginiti, James Peter, 5180 Foster Road, Canandaigua, New York 14424, (US)
Anilionis, Algis, 25 Whistlerhill Lane, Huntington, New York 11743, (US)
LEGAL REPRESENTATIVE:
Wachtershauser, Gunter, Prof. Dr. (12711), Patentanwalt, Tal 29, 80331 Munchen, (DE)

PATENT (CC, No, Kind, Date): EP 512260 A2 921111 (Basic)
EP 512260 A3 930728
EP 512260 B1 010704
APPLICATION (CC, No, Date): EP 92105887 920406;
PRIORITY (CC, No, Date): US 695706 910503
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LI; LU; NL; PT; SE
INTERNATIONAL PATENT CLASS: C12N-015/74; A61K-039/112; C12N-015/74; C12R-1:42

ABSTRACT EP 512260 A2
This invention pertains to a complementation system for the selection and maintenance of expressed genes in bacterial hosts. The invention provides stable *vectors"** which can be selected and maintained by complementation of chromosomal deletion mutations of purA (adenylosuccinate synthetase), obviating the use of antibiotic resistance genes. This system is useful in production organisms during fermentation and in live vaccine bacteria, such as *attenuated"** *Salmonella"** *typhi"**. This system allows for selection of chromosomal integrants and for selection and stable *plasmid"** maintenance in the vaccinated host without application of external selection pressure.
ABSTRACT WORD COUNT: 92

NOTE:
Figure number on first page: NONE

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	EPABF1	514
CLAIMS B	(English)	200127	1730
CLAIMS B	(German)	200127	1667
CLAIMS B	(French)	200127	2184
SPEC A	(English)	EPABF1	8964
SPEC B	(English)	200127	8872
Total word count - document A			9479
Total word count - document B			14453
Total word count - documents A + B			23932

17/3,AB/27 (Item 15 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

00492830
Recombinant immunogenic actinomycetale.
Immunogene rekombinante Actinomycetale.
Actinomycetale immunogene recombinant.
PATENT ASSIGNEE:
INSTITUT PASTEUR, (250790), 25-28, rue du Docteur Roux, F-75724 Paris Cedex 15, (FR), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;GR;IT;LI;LU;NL;SE)
INVENTOR:
Gicquel, Brigitte, 8 rue Daguerre, F-75014 Paris, (FR)
Winter, Nathalie, 46 rue d'Avron, F-75020 Paris, (FR)
Gheorghiu, Marina, 10 rue Charcot, F-92200 Neuilly Sur Seine, (FR)
LEGAL REPRESENTATIVE:
Gutmann, Ernest et al (15992), S.C. Ernest Gutmann - Yves Plasseraud 67, boulevard Haussmann, F-75008 Paris, (FR)
PATENT (CC, No, Kind, Date): EP 521220 A1 930107 (Basic)
APPLICATION (CC, No, Date): EP 91401601 910614;
PRIORITY (CC, No, Date): EP 91401601 910614
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LI; LU; NL; SE
INTERNATIONAL PATENT CLASS: C12N-015/74; A61K-039/04;
ABSTRACT EP 521220 A1
A mycobacteria transformed with an antigen-encoding gene, such as nef, under the control of a Streptomyces stress-responsive promoter, such as the S. albus groES/groEL1 promoter, and preferably associated with a synthetic ribosome binding site. The recombinant mycobacteria can be used as a vaccine against, for example, a pathogen which carries the antigen.
ABSTRACT WORD COUNT: 54

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	EPABF1	332
SPEC A	(English)	EPABF1	5955
Total word count - document A			6287
Total word count - document B			0
Total word count - documents A + B			6287

17/3,AB/28 (Item 16 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

00468674
Insertion of DNA by modified transposons.
Einfugen von DNS mittels abgewandelter Transposons.
Insertion d'ADN au moyen de transposones modifies.
PATENT ASSIGNEE:
AMERICAN CYANAMID COMPANY, (212591), 1937 West Main Street P.O. Box 60, Stamford Connecticut 06904-0060, (US), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;GR;IT;LI;LU;NL;SE)
INVENTOR:
Brey, Robert Newton, III, 74 Sagamore Drive, Rochester, New York 14617, (US)
Deich, Robert Allen, 10 Fallbrook Circle, Rochester, New York 14625, (US)

LEGAL REPRESENTATIVE:
Wachtershauser, Gunter, Dr. (12711), Tal 29, W-8000 Munchen 2, (DE)
PATENT (CC, No, Kind, Date): EP 485701 A1 920520 (Basic)
APPLICATION (CC, No, Date): EP 91114668 910830;
PRIORITY (CC, No, Date): US 590364 900928
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LI; LU; NL; SE
INTERNATIONAL PATENT CLASS: C12N-015/63; C12N-015/90; A61K-039/00; C12N-015/70; C12N-015/74; C12N-015/30; C12N-015/31
ABSTRACT EP 485701 A1

DNA constructs for the introduction of a DNA sequence into the constituent DNA of a prokaryote, and methods of use. The DNA construct includes an expressible gene encoding a transposase protein, linked in cis to a transposable cassette. The transposable cassete includes a pair of transposase recognition sequences flanking the DNA sequence. The gene encoding the transposase protein is not flanked by the transposase recognition sequences.

ABSTRACT WORD COUNT: 68

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	EPABF1	639
SPEC A	(English)	EPABF1	11212
Total word count - document A			11851
Total word count - document B			0
Total word count - documents A + B			11851

17/3,AB/29 (Item 17 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

00367597
Salmonella transformant capable of expression of heterologous genes and useful as a recombinant vaccine.
Salmonella-Transformant, fahig zur Expression von heterologen Genen, verwendbar als rekombinantes Vakzin.
Transformant de salmonella, capable d'expression de genes heterologues, utile comme vaccin recombinant.
PATENT ASSIGNEE:
SMITHKLINE BECKMAN CORPORATION, (201242), One Franklin Plaza P O Box 7929 , Philadelphia Pennsylvania 19103, (US), (applicant designated states: AT;BE;CH;DE;ES;FR;GB;GR;IT;LI;LU;NL;SE)
UNITED STATES OF AMERICA as represented by THE SECRETARY OF THE ARMY, (991611), Department of Army U.S. Army Legal Services Agency Nassif Building, Falls Church, VA 22041-5013, (US), (applicant designated states: AT;BE;CH;DE;ES;FR;GB;GR;IT;LI;LU;NL;SE)
INVENTOR:
Sadoff, Jerald Charles, 1622 Kalmia Road, Washington, DC 20012, (US)
Young, James Francis, 12624 Gravenhurst Lane, Gaithersburg MD 20878, (US)
LEGAL REPRESENTATIVE:
Giddings, Peter John, Dr. et al (55331), Smith Kline & French Laboratories Ltd. Corporate Patents Mundells, Welwyn Garden City Hertfordshire AL7 1EY, (GB)
PATENT (CC, No, Kind, Date): EP 357208 A1 900307 (Basic)
APPLICATION (CC, No, Date): EP 89307406 890720;
PRIORITY (CC, No, Date): US 222202 880721
DESIGNATED STATES: AT; BE; CH; DE; ES; FR; GB; GR; IT; LI; LU; NL; SE

INTERNATIONAL PATENT CLASS: A61K-039/112; C12N-015/00; A61K-039/015; C12N-015/00; C12R-001/42; C12N-015/00; C12R-001/90

ABSTRACT EP 357208 A1
The invention provides a Salmonella baceterial strain transformed with an expression *vector"** comprising a heterologous gene operatively linked to an E. coli promoter sequence, the transformed strain being capable of constitutive expression of the product of the heterologous gene. Also provided is an attenuated Salmonella strain containing all or a portion of a selected circumsporozoite protein, useful for inducing protective cell-mediated immunity to malarial parasites.
ABSTRACT WORD COUNT: 69

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	EPABF1	812
SPEC A	(English)	EPABF1	5526
Total word count - document A			6338
Total word count - document B			0
Total word count - documents A + B			6338

17/3,AB/30 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Resource

0260177 DBA Accession No.: 2000-14667
In vivo correction of genetic defects of monocyte/macrophages using attenuated Salmonella as oral *vectors"** for targeted gene delivery - *plasmid"** pCNV-beta and *plasmid"** pCG3-mediated *beta"**-*galactosidase"** and interferon-gamma gene transfer for infection nucleic acid vaccine and gene therapy
AUTHOR: Paglia P; Terrazzini N; Schulze K; Guzman C A; Colombo M P
CORPORATE AFFILIATE: Nat.Cancer-Inst.Milan Ges.Biotechnol.Forsch.
CORPORATE SOURCE: Immunotherapy and Gene Therapy Unit, Department of Experimental Oncology, Istituto Nazionale Tumori, Via Venezian 1, 20133 Milan, Italy.
JOURNAL: Gene Ther. (7, 20, 1725-30) 2000
ISSN: 0969-7128 CODEN: GETHEC
LANGUAGE: English
ABSTRACT: Correction of a genetic disorder associated with monocytes and macrophages using *attenuated"** *Salmonella"** *typhimurium"** aroA-strain *SL7207"** as a DNA delivery system was investigated. Peritonal unelicited mouse macrophages were infected with SL7207 or its isogenic derivative carrying *plasmid"** *pCMV"**-beta containing the *beta"**-*galactosidase"** (EC-3.2.1.23) gene. 90% Of the macrophages infected with the *plasmid"** expressed the gene after 48 hr. SL7207 was transformed with *plasmid"** pCG3 that contained the gene coding the mouse interferon-gamma (mIFN-gamma) and was used to infect, in vitro mouse peritoneal macrophages obtained from mice knocked out for the IFN-gamma gene. Recombinant SL7207 carrying either the *pCMV"**-beta or pC3G were administered by injection to GKO mice. Release of IFN-gamma was detected in the GKO mice receiving the *SL7207"**(pCG3) strain, confirming *attenuated"** *S17207"** can be used in vivo as a *vector"** for gene therapy. Oral administration of S17207 resulted in targeted gene expression within macrophages to replenish their immunological functions and re-established the natural resistance to

bacterial infection. (30 ref)

17/3,AB/31 (Item 2 from file: 357)
DIALOG(R)File 357:Derwent Biotech Resource

0239173 DBA Accession No.: 99-09274
Salmonella vaccine carrier strains: effective delivery system to trigger antitumor immunity by oral route - oral immunization of Salmonella typhimurium transformed with promoter-controlled *beta"**-*galactosidase"**, may be useful for prevention of cancer
AUTHOR: Medina E; +Guzman C A; Staendner L H; Colombo M P; Paglia P
CORPORATE AFFILIATE: Ges.Biotechnol.Forsch. Nat.Tumor-Inst.Milan
CORPORATE SOURCE: Division of Microbiology, GBF-National Research Centre for Biotechnology, Mascheroder Weg 1, D-38124 Brunswick, Germany. email:cag@gbf.de
JOURNAL: Eur.J.Immunol. (29, 2, 693-99) 1999
ISSN: 0014-2980 CODEN: EJIMAF
LANGUAGE: English
ABSTRACT: The potential of attenuated Salmonella spp. to trigger antitumor immunity was investigated using *beta"**-*galactosidase"** (I) (EC-3.2.1.23) as a model tumor-associated antigen (TAA). (I) was expressed in a Salmonella typhimurium aroA strain SL7207 vaccine carrier strain either constitutively using *plasmid"** pAH97, or under the control of a promoter using *plasmid"** pLS1000 following infection. When either vaccine was used for oral immunization of BALB/c mice a (I)-specific humoral and cell-mediated immunity resulted. Antigen-specific cytotoxic T-lymphocyte responses were more efficient when the expression was controlled by the promoter which was activated upon infection. When mice afflicted with an aggressive fibrosarcoma were transfected with (I) (which operationally acts as a TAA), vaccinated mice exhibited a significant reduction in tumor take and growth in comparison to mice vaccinated with a plasmidless SL7207 strain. Again, the overall efficiency was better in the group in which (I) was controlled by the in vivo-activated promoter (85% versus 54%). (40 ref)

17/3,AB/32 (Item 3 from file: 357)
DIALOG(R)File 357:Derwent Biotech Resource

0230897 DBA Accession No.: 99-00998 PATENT
Oral contraceptive vaccine containing recombinant Salmonella - *plasmid"** pMOhly1 containing a zona pellucida protein antigen
AUTHOR: Donner P; Goebel W; Demuth A; Gentschev I; Hess J; Kaufmann S
CORPORATE SOURCE: Berlin, Germany.
PATENT ASSIGNEE: Schering-Berlin 1998
PATENT NUMBER: DE 19720761 PATENT DATE: 981112 WPI ACCESSION NO.: 98-596140 (9851)
PRIORITY APPLIC. NO.: DE 1020761 APPLIC. DATE: 970507
NATIONAL APPLIC. NO.: DE 1020761 APPLIC. DATE: 970507
LANGUAGE: German
ABSTRACT: The use of a *vector"**, *plasmid"** pMOhly1, for expression and secretion of a fertility control antigen, zona pellucida protein, in *attenuated"** *Salmonella"** *typhimurium"** or other *attenuated"** Gram-negative vaccine strains to produce an oral vaccine is new. The

*vector"** contains a gene encoding the fertility control antigen under the control of a complete hemolysin operon, including the *hly"** specific promoter and the hlyR enhancer-like regulator but excluding most of the hlyA gene. The *vector"** is used for immunological contraception by oral administration. In an example, huZPB-1, a 207 bp cDNA fragment (from nucleotides 127-333 of human zona pellucida gene-B) was amplified from *plasmid"** pGEX-KG-huZPB and inserted into pMOhly1. Transformation of S. typhimurium SL7207 with pMOhly1 resulted in secretion of a 19 kD zona pellucida B/hemolysin fusion protein that should induce a mucosal immune response to ZPB. (17pp)

17/3,AB/33 (Item 4 from file: 357)
DIALOG(R)File 357:Derwent Biotech Resource

0230892 DBA Accession No.: 99-00993 PATENT
Attenuated Salmonella strain carrying eukaryotic *vectors"** expressing heterologous/autologous genes - *vector"** *plasmid"** *pCMV"** -mediated eukaryotic gene transfer and expression in e.g. Salmonella typhimurimu or Salmonella tyhii for application as a nucleic acid vaccine
AUTHOR: Darji A; Guzman C; Timmis K; Wehland J; Weiss S; Gerstel B; Chakraborty T; Wachholz P
CORPORATE SOURCE: Brunswick, Germany.
PATENT ASSIGNEE: Ges.Biotechnol.Forsch. 1998
PATENT NUMBER: WO 9848026 PATENT DATE: 981029 WPI ACCESSION NO.: 98-609995 (9851)
PRIORITY APPLIC. NO.: DE 4106503 APPLIC. DATE: 970418
NATIONAL APPLIC. NO.: WO 97DE6933 APPLIC. DATE: 971211
LANGUAGE: English
ABSTRACT: An *attenuated"** Salmonella strain (preferably *Salmonella"** *typhimurimu"** aroA544, ATCC 33275 or Salmonella tyhii Ty21a) carries a eukaryotic *vector"** for expressing a heterologous/ autologous gene in Escherichia coli (e.g. *beta"**-*galactosidase"**, EC-3.2.1.23) or gene fragment within an open reading frame inside the *vector"** (*plasmid"** *pCMV"**) is new. The attenuation is adjusted to the vaccination of vertebrate including humans. Also claimed are: a recovery process for the attenuated Salmonella strain; a vaccine for oral and/or nasal gene delivery containing the Salmonella strain; and an immunogenic protein or protective antigen expressed by the *vector"** , where, a gene or gene fragment from a heterologous/autologous DNA.cDNA library is expressed by a eukaryotic expression *vector"** carried by an attenuated Salmonella strain, a DNA nucleic acid vaccination performed using the Salmonella strain, screening for an expressed product is performed according to above providing an immune response and recovery of the Salmonella strain, immunogenic protein or protective antigen is performed. The strain can be used as a nucleic acid vaccine and for oral, nasal or mucosal gene transfer to humans. (52pp)

17/3,AB/34 (Item 5 from file: 357)
DIALOG(R)File 357:Derwent Biotech Resource

0185720 DBA Accession No.: 95-12541 PATENT
New DNA constructs containing the htrA promoter - htrA promoter-containing

DNA construct preparation; linkage to heterologous fusion protein DNA sequence; attenuated bacterium transformation for vaccine composition production
AUTHOR: Khan M A; Chatfield S N; Li J
PATENT ASSIGNEE: Medeva-Holdings 1995
PATENT NUMBER: WO 9520665 PATENT DATE: 950803 WPI ACCESSION NO.: 95-275450 (9536)
PRIORITY APPLIC. NO.: GB 941795 APPLIC. DATE: 940131
NATIONAL APPLIC. NO.: WO 95GB196 APPLIC. DATE: 950131
LANGUAGE: English
ABSTRACT: A DNA construct (*plasmid"** pHTRA2) containing a htrA promoter sequence operably linked to a DNA sequence encoding one or more heterologous fusion proteins is new The proteins making up the fusion are linked by a flexible hinge region. The first heterologous protein is an antigenic sequence containing tetanus toxin fragment-C or one or more epitopes of it. A replicable expression *vector"** containing a DNA construct suitable for use in bacteria, and a host cell containing a DNA construct in a chromosomal or extra-chromosomal form are also claimed. The htrA promoter is induced by temp. increases and is therefore inducible in vivo to produce heterologous proteins. The DNA construct can be used to express a protein or to transform an attenuated bacterium to produce vaccine compositions which can be used for the treatment or prevention of human infections. In an example, a DNA construct containing a htrA promoter and a *lacZ"** gene was used to transform Salmonella typhimurium. The transformed bacteria were grown at 30 deg and temp. shifts from 30-37 deg resulting in an increase in *beta"**-*galactosidase"** (EC-3.2.1.23) enzyme units due to the induction of the htrA promoter. (54pp)

17/3,AB/35 (Item 6 from file: 357)
DIALOG(R)File 357:Derwent Biotech Resource

0178324 DBA Accession No.: 95-05145
Immunization with live recombinant Salmonella typhimurium aroA producing F1 antigen protects against plague - using *vector"** *plasmid"** pFGAL2a, for application as a live, attenuated, oral recombinant vaccine
AUTHOR: Oyston P C F; Williamson E D; Leary S E C; Eley S M; Griffin K F; Titball R W
CORPORATE AFFILIATE: Chem.Biol.Def.Estab.Porton-Down
CORPORATE SOURCE: Chemical and Biological Defense Establishment, Porton Down, Salisbury, Wiltshire, SP4 0JQ, UK.
JOURNAL: Infect.Immun. (63, 2, 563-68) 1995
ISSN: 0019-9567 CODEN: INFIBR
LANGUAGE: English
ABSTRACT: A DNA fragment from Yersinia pestis that encoded the open reading frame of the capsular antigen F1 gene (caf1) without its signal sequence, was amplified, purified, digested with SacI and AccI, and cloned into *plasmid"** pUC18 (which contained the caf1 gene without the signal peptide) to form an in-frame fusion with the initial 17 amino acids of *beta"**-*galactosidase"** (EC-3.2.1.23) expressed from the lac promoter. This construct, *plasmid"** pFGAL2a was purified and electroporated into aroAhis mutant Salmonella typhimurium SL3261 and F1 expression was examined by SDS-PAGE. Male BALB/c mice were vaccinated i.t. and i.v. with S. typhimurium/pGAL2a constructs (50 million) in 200 ul of phosphate buffered saline. The immunity induced was able to protect mice against challenge with a virulent strain of plague. The F1

antigen of Y. pestis was expressed in the attenuated Salmonella strain and it induced 90% protection against challenge by a virulent Y. pestis strain. Protection correlated with the induction of high titers of IgG in serum samples and specific T-lymphocyte response. This method shows potential as the basis for a live, attenuated oral vaccine. (36 ref)

17/3,AB/36 (Item 7 from file: 357)
DIALOG(R)File 357:Derwent Biotech Resource

0136604 DBA Accession No.: 92-09096
Salmonella as a vaccine *vector"**: controlled de novo expression of antigen in mice for immunization with toxic proteins - Salmonella typhimurium use as *vector"** for toxic antigen expression in e.g. mouse (conference abstract)
AUTHOR: Ervin S E; Small Jr P A; Gulig P A
CORPORATE SOURCE: University of Florida College of Medicine, Gainesville, FL 32611, USA.
JOURNAL: Abstr.Gen.Meet.Am.Soc.Microbiol. (92 Meet., 158) 1992
CODEN: 0005P
LANGUAGE: English
ABSTRACT: An expression system was previously developed in *attenuated"** *Salmonella"** *typhimurium"** for toxic antigens which are repressed in vitro but are induced in vivo after oral immunization of mice. Another system was reported in which *beta"**-*galactosidase"** (bG, EC-3.2.1.23) was used as a model antigen. The *lacZ"** gene was expressed from the trc promoter on one *plasmid"** and was repressed by lacIq in trans on a second. The repressor *plasmid"** was incompatible with the expression *plasmid"** , so that segregation occurred with bacterial growth resulting in induction of *lacZ"**. Expression of antigen in vitro increased 10-fold after 20 generations and correlated with segregation of the repressor *plasmid"** . Mice were orally inoculated with *attenuated"** *S"**. *typhimurium"** containing both *plasmids"**. The *S"**. *typhimurium"** carrier was *attenuated"** by curing of the virulence *plasmid"**. The incompatible repressor *plasmid"** segregated in vivo and in vitro resulting in anti-bG IgG antibody levels as high as those achieved with an unrepressed expression system. The system may be used to deliver toxic antigens to induce both systemic and mucosal immunity. (0 ref)

17/3,AB/37 (Item 8 from file: 357)
DIALOG(R)File 357:Derwent Biotech Resource

0114937 DBA Accession No.: 91-02579
Synthesis of the surface glycoprotein of rota virus-SA11 in the aroA strain of Salmonella typhimurium SL3261 - potential application in recombinant vaccine construction (conference paper)
AUTHOR: Salas-Vidal E; Plebanski M; Castro S; Perales G; Mata E; Lopez S
CORPORATE SOURCE: Centro de Investigaciones sobre Ingenieria Genetica y Biotecnologia, Universidad Nacional Autonoma de Mexico, Ap. Postal 510-3, Cuernavaca, Morelos 62271, Mexico.
JOURNAL: Res.Microbiol. (141, 7-8, 883-86) 1990
CODEN: RMCREW
LANGUAGE: English

ABSTRACT: The possibility of constructing a rota virus recombinant vaccine eliciting a mucosal immune response was investigated. 2 Protein antigens, VP4 (mol.wt. 88,000) and VP7 (mol.wt. 37,000) are capable of inducing neutralizing antibodies in the host. A recombinant VP7 (86%) and *beta"**-*galactosidase"** (EC-3.2.1.23) fusion protein was produced in Escherichia coli, and the fusion protein induced neutralizing antibodies in mice. *Attenuated"** aroA *Salmonella"** *typhimurium"** SL 3261 was used as host for fusion protein production. *Plasmid"** pUMA93Z was modified by insertion of the rop gene (*plasmid"** pUMA93Z-rop), which decreases the *plasmid"** copy number, and was used for transfection of S. typhimurium. Recombinant strains with pUMA93Z and pUMA93Z-rop produced the fusion protein to 1% and 0.5% of total protein, respectively. After i.v. inoculation into mice, stability depended on the level of fusion protein production and the *plasmid"** copy number. Although *beta"**-*galactosidase"** antibodies were produced, no rota virus antibodies were detected. Improved immunogenicity of the antigen and improved *plasmid"** stability in the host should allow improved vaccine construction. (3 ref)

17/3,AB/38 (Item 9 from file: 357)
DIALOG(R)File 357:Derwent Biotech Resource

0114935 DBA Accession No.: 91-02577
Preliminary studies on infection by attenuated Salmonella in guinea-pig and on expression on herpes simplex virus - glycoprotein-D-1 antigen gene cloning in *attenuated"** *Salmonella"** *typhimurium"**; potential application in recombinant vaccine construction (conference paper)
AUTHOR: Bowen J C; Alpar O; Phillpotts R; Roberts I S; Brown M R W
CORPORATE SOURCE: Pharmaceutical Sciences Institute, Aston University, Aston Triangle, Birmingham B4 7ET, UK.
JOURNAL: Res.Microbiol. (141, 7-8, 873-77) 1990
CODEN: RMCREW
LANGUAGE: English
ABSTRACT: A herpes simplex virus glycoprotein-D-1 (gD-1) gene was cloned and expressed in aroA-*attenuated"** *Salmonella"** *typhimurium"**, under the control of an Escherichia coli *lacZ"** promoter. A 1 kb fragment from the gD gene was amplified using the polymerase chain reaction, inserted in the BamHI site of *plasmid"** pUC19 (to form *plasmid"** pJB1) and cloned in Escherichia coli LE392 (*plasmid"** pMW1). The recombinants showed improved *plasmid"** stability, and the recombinant gD protein was detected by colony immunoblotting and ELISA. In E. coli LB5010, *plasmid"** pJB1 appeared stable in the absence of *plasmid"** pMW1. Transduction of *plasmid"** pJB1 into *attenuated"** *S"**. *typhimurium"** strains was carried out, for future evaluation in a guinea-pig animal model. A guinea-pig vaginal challenge model was tested using host S. typhimurium aroA strains, and successful colonization was achieved, indicating that this system is suitable for testing a potential recombinant vaccine against herpes simplex virus. (18 ref)

Set	Items	Description
S18	90	AU=(DARJI A? OR DARJI, A?)
S19	529	AU=(GUZMAN, C? OR GUZMAN C?)
S20	757	AU=(TIMMIS, K? OR TIMMIS K?)
S21	4702	AU=(WEISS, S? OR WEISS S?)
S22	27	AU=(GERSTEL, B? OR GERSTEL B?)

- Authors

Searcher : Shears 308-4994

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S23        823   AU=(CHAKRABORTY, T? OR CHAKRABORTY T?)
S24         13   AU=(WACHHOLZ, P? OR WACHHOLZ P?)
S25          2   S18 AND S19 AND S20 AND S21 AND S22 AND S23 AND S24
S26         47   S18 AND (S19 OR S20 OR S21 OR S22 OR S23 OR S24)
S27         79   S19 AND (S20 OR S21 OR S22 OR S23 OR S24)
S28         13   S20 AND (S21 OR S22 OR S23 OR S24)
S29         47   S21 AND (S22 OR S23 OR S24)
S30         18   S22 AND (S23 OR S24)
S31          3   S22 AND S24
S32         13   (S18 OR S19 OR S20 OR S21 OR S22 OR S23 OR S24 OR S27 OR S-
                 26 OR S29) AND S13
S33         26   (S25 OR S28 OR S30 OR S31 OR S32) NOT S16
S34         13   RD (unique items)
>>>No matching display code(s) found in file(s): 65, 113

 34/3,AB/1     (Item 1 from file: 65)
DIALOG(R)File  65:Inside Conferences
(c) 2002 BLDSC all rts. reserv. All rts. reserv.

01421054   INSIDE CONFERENCE ITEM ID: CN014100611
Analysis of the Interaction of the Actin Nucleating Factor ActA  From
Listeria monocytogenes with the Actin Microfilament System of  Eukaryotic
Cells
  Pistor, S.; *Chakraborty, T."**; Niebuhr, K.; *Gerstel, B."**
  CONFERENCE: Problems of listeriosis-International symposium; 12th
  PROCEEDINGS OF THE INTERNATIONAL SYMPOSIUM ON PROBLEMS OF  LISTERIOSIS,
  1995; 12th  P: 335-336
  Canning Bridge, Promaco Conventions Pty Ltd, 1995
  ISBN: 1863080406
  LANGUAGE: English  DOCUMENT TYPE: Conference Papers and abstracts
    CONFERENCE LOCATION: Perth, Australia
    CONFERENCE DATE:  Oct 1995 (19951) (19951)
  NOTE:
    Also known as ISOPOL XII


 34/3,AB/2     (Item 1 from file: 77)
DIALOG(R)File  77:Conference Papers Index
(c) 2002 Cambridge Sci Abs. All rts. reserv.

Supplier Accession Number: 82035979           V10N7
  No More Animal Experiments in Demonstration of Enterotoxins
  Chakraborty, T.; Helmuth, R.; Timmis, K.; Sanyal, S.C.; Bulling, E.
  Fed. Hlth. Off., Inst. Veterinary Med., Berlin, FRG
  XIII International Congress of Microbiology  8230030  Boston, MA  8-13
Aug 82
  International  Union  of  Microbiological Societies; American Society for
Microbiology
  1982,  Program  and  abstracts  book  available:  American  Society  for
Microbiology,  1913 I St. NW, Washington, DC 20006, ISBN 0-914826-44-1; 182
pages; Price: $10 Poster P34:3


 34/3,AB/3     (Item 1 from file: 144)
DIALOG(R)File 144:Pascal
(c) 2002 INIST/CNRS. All rts. reserv.

  12594101   PASCAL No.: 96-0280474
```

The ActA polypeptides of Listeria ivanovii and Listeria monocytogenes Harbor related binding sites for host microfilament proteins

*GERSTEL B"**; GROEBE L; PISTOR S; *CHAKRABORTY T"**; WHELAND J; SANSONETTI P J ed

Abteilung Zellbiologie und Immunologie, Gesellschaft fuer Biotechnologie Forschung, 38124 Brauschweig, Germany

Journal: Infection and immunity, 1996, 64 (6) 1929-1936

Language: English

The surface-bound ActA polypeptide of the intracellular bacterial pathogen Listeria monocytogenes acts as a nucleator protein, generating the actin cytoskeleton around intracellularly motile bacteria. In this work, we examined the functional similarity of ActA from Listeria ivanovii (iActA) ATCC 19119 to its L. monocytogenes counterpart. The amino acid sequence of iActA predicts a molecular mass of 123 kDa and harbors eight proline-rich repeats. For functional analysis, various iActA derivatives and hybrid constructs ofL. ivanovii and L. monocytogenes ActA polypeptides were transiently expressed in epithelial cells and examined for recruitment of host microfilament proteins by a mitochondrial targeting assay. As has been demonstrated with ActA, iActA also spontaneously inserted into the surface of mitochondria and induced recruitment of actin, alpha -actinin, and the vasodilator-stimulated phosphoprotein (VASP) to these subcellular organelles. By comparison of amino-terminally truncated iActA derivatives for their ability to recruit cytoskeletal proteins, a region essential for actin filament accumulation was identified between amino acid residues 290 and 325. Such derivatives, however, retained their ability to bind VASP. Replacement of the proline-rich repeats in ActA with those of iActA also resulted in VASP recruitment. Hence, despite the limited overall sequence homology between ActA and iActA, the two molecules consist of at least two similar domains : a highly positively charged N-terminal domain that is directly involved in actin filament recruitment and a proline-rich repeat region required for VASP binding.

34/3,AB/4 (Item 2 from file: 144)
DIALOG(R)File 144:Pascal

05967688 PASCAL No.: 85-0153033

Cloning of enterotoxin gene from Aeromonas hydrophila provides conclusive evidence of production of a cytotonic enterotoxin

*CHAKRABORTY T"**; MONTENEGRO M A; SANYAL S C; HELMUTH R; BULLING E; *TIMMIS K N"**

Bundesgesundheitsamt, Berlin, Federal Republic of Germany

Journal: Infection and immunity, 1984, 16 (2) 435-441

Language: English

Afin de resoudre certaines controverses concernant l'enterotoxine de A. hydrophila, les auteurs ont exploite la resistance moderee a l'inactivation par chaleur de la toxine et les methodes de clonage des genes afin d'obtenir l'evidence que les enterotoxines de A. hydrophila AH2 et AH1133 sont cytotoniques et que les activites enterotoxique, cytotoxique et hemolotique de ces souches sont distincts et sont determinees par des genes differents

34/3,AB/5 (Item 1 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

12073320 GENUINE ARTICLE#: 362TV NUMBER OF REFERENCES: 47
TITLE: Mutations of arginine residues within the 146-KKRRK-150 motif of the ActA protein of Listeria monocytogenes abolish intracellular motility by interfering with the recruitment of the Arp2/3 complex
AUTHOR(S): Pistor S (REPRINT); Grobe L; Sechi AS; Domann E; *Gerstel B"**; Machesky LM; *Chakraborty T"**; Wehland J
AUTHOR(S) E-MAIL: spi@biobase.de
CORPORATE SOURCE: Gesell Biotechnol Forsch GmbH, Dept Cell Biol, Mascheroder Weg 1/D-38124 Braunschweig//Germany/ (REPRINT); Gesell Biotechnol Forsch GmbH, Dept Cell Biol, /D-38124 Braunschweig//Germany/ ; Univ Giessen, Inst Med Mikrobiol, /D-35392 Giessen//Germany/; Univ Birmingham, Dept Biochem, /Birmingham B15 2TT/W Midlands/England/
PUBLICATION TYPE: JOURNAL
PUBLICATION: JOURNAL OF CELL SCIENCE, 2000, V113, N18 (SEP), P3277-3287
PUBLISHER: COMPANY OF BIOLOGISTS LTD, BIDDER BUILDING CAMBRIDGE COMMERCIAL PARK COWLEY RD, CAMBRIDGE CB4 4DL, CAMBS, ENGLAND
ISSN: 0021-9533
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: The recruitment of actin to the surface of intracellular Listeria monocytogenes and subsequent tail formation is dependent on the expression of the bacterial surface protein ActA, Of the different functional domains of ActA identified thus far, the N-terminal region is absolutely required for actin filament recruitment and intracellular motility, Mutational analysis of this domain which abolished actin recruitment by intracellular Listeria monocytogenes identified two arginine residues within the 146-KKRRK-150 motif that are essential for its activity. More specifically, recruitment of the Arp2/3 complex to the bacterial surface, as assessed by immunofluorescence staining with antibodies raised against the p21-Arc protein, was not obtained in these mutants. Consistently, treatment of infected cells,vith latrunculin B, which abrogated actin filament formation, did not affect association of ActA,vith p21-Arc at the bacterial surface. Thus, the initial recruitment of the Arp2/3 complex to the bacterial surface is independent of, and precedes, actin polymerisation, Our data suggest that binding of the Arp2/3 complex is mediated by specific interactions dependent on arginine residues within the 146-KKRRK-150 motif present in ActA.

34/3,AB/6 (Item 2 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

10317692 GENUINE ARTICLE#: 169FF NUMBER OF REFERENCES: 45
TITLE: Pathogenicity island 2 mutants of Salmonella typhimurium are efficient carriers for heterologous antigens and enable modulation of immune responses
AUTHOR(S): Medina E; Paglia P; Nikolaus T; Muller A; Hensel M; *Guzman CA (REPRINT)"**
AUTHOR(S) E-MAIL: cag@gbf.de
CORPORATE SOURCE: GBF, Div Microbiol, Mascheroder Weg 1/D-38124 Braunschweig//Germany/ (REPRINT); GBF, Div Microbiol, /D-38124 Braunschweig//Germany/; Max Von Pettenkofer Inst Hyg & Med Microbiol, Lehrstuhl Bakteriol, /D-80336 Munich//Germany/; Ist Nazl Studio & Cura Tumori, Expt Immunotherapy & Gene Therapy Unit, /I-20133 Milan//Italy/
PUBLICATION TYPE: JOURNAL
PUBLICATION: INFECTION AND IMMUNITY, 1999, V67, N3 (MAR), P1093-1099
PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW,

WASHINGTON, DC 20005-4171 USA
ISSN: 0019-9567
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: The potential use as vaccine delivery system of Salmonella typhimurium strains harboring defined mutations in the sseC (HH104) and sseD (MvP101) genes, which encode putative effector proteins of the type III secretion system of Salmonella pathogenicity island 2, was evaluated and compared with that of the well-characterized aroA mutant strain SL7207 by using *beta"**-*galactosidase"** (*beta"**-*Gal"**) as a model antigen. When orally administered to immune-competent or gamma interferon-deficient (TFN-gamma(-/-)) BALB/c mice, both mutants were found to be highly attenuated (50% lethal dose, >10(9) bacteria). Both strains were also able to efficiently colonize and persist in Peyer's patches. Immunization with HH104 and MvP101 triggered *beta"**-*Gal"** -specific serum and mucosal antibody responses equivalent to or stronger than those observed in SL7207-immunized mice. Although immunoglobulin G2 (IgG2) serum antibodies were dominant in all groups, IgG1 was also significantly increased in mice vaccinated with MvP101 and SL7207. Comparable *beta"**-*Gal"**-specific IgA and Ige antibodies were detected in intestinal lavages from mice immunized with the different strains. Antigen-specific CD4(+) T-helper cells were generated after vaccination with all vaccine prototypes; however, responses were significantly more efficient when HH104 and MvP101 were used ($P < 0.05$). Significantly higher levels of IFN-gamma were produced by restimulated spleen cells from mice immunized with HH104 than from those vaccinated with the MvP101 or SL7207 derivatives (P less than or equal to 0.05). Interestingly, the three strains induced major histocompatibility complex class I-restricted CD8(+) cytotoxic T cells against *beta"**-*Gal"**; however, cytotoxic T-lymphocyte responses were significantly stronger after immunization with HH104 ($P < 0.05$). These novel *S"**. *typhimurium"** *attenuated"** strains constitute promising delivery systems for vaccine antigens. The qualitative differences observed in the obtained responses with different carriers may be useful for those applications in which a targeted immunomodulation is required.

34/3,AB/7 (Item 3 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

09779372 GENUINE ARTICLE#: 112UB NUMBER OF REFERENCES: 35
TITLE: The role of the bacterial membrane protein ActA in immunity and protection against Listeria monocytogenes
AUTHOR(S): Darji A (REPRINT); Bruder D; ZurLage S; *Gerstel B"**; *Chakraborty T"**; Wehland J; Weiss S
CORPORATE SOURCE: GESELL BIOTECHNOL FORSCH MBH,DIV CELL BIOL & IMMUNOL, MASCHERODER WEG 1/D-38124 BRAUNSCHWEIG//GERMANY/ (REPRINT); UNIV GIESSEN,INST MED MICROBIOL/GIESSEN//GERMANY/
PUBLICATION TYPE: JOURNAL
PUBLICATION: JOURNAL OF IMMUNOLOGY, 1998, V161, N5 (SEP 1), P2414-2420
PUBLISHER: AMER ASSOC IMMUNOLOGISTS, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814
ISSN: 0022-1767
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: ActA, an essential virulence factor of Listeria monocytogenes, is an integral membrane protein that is required for intracellular motility, cell-to-cell spread, and rapid dissemination of the bacteria

in the infected host. To reveal cytotoxic T cell responses against ActA we introduced a recombinant soluble form of ActA into the MHC class I-processing compartment of APC using a variant of listeriolysin mutated within its immunodominant MHC class I epitope. With this experimental system we demonstrate that T cells are induced against ActA during a sublethal infection with L, monocytogenes. However, adoptively transferred cytotoxic CD8(+) T cells specific for ActA did not protect mice against a subsequent challenge with this pathogen, This was due to an inability of APC to present ActA by either MHC class I or class II molecules as long as ActA remained tethered to the surface of intracellular viable bacteria, ActA was only presented when L. monocytogenes were engineered to secrete ActA or when the bacteria were killed by antibiotics during the assay. These findings raise questions on the general use of membrane proteins of pathogens as candidates for subunit vaccines.

34/3,AB/8 (Item 4 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

09578074 GENUINE ARTICLE#: ZV003 NUMBER OF REFERENCES: 30
TITLE: Attenuated Listeria monocytogenes carrier strains can deliver an HIV-1 gp120 T helper epitope to MHC class II restricted human CD4(+) T cells
AUTHOR(S): Guzman CA (REPRINT); Saverino D; Medina E; Fenoglio D; *Gerstel B"**; Merlo A; Pira GL; Buffa F; *Chakraborty T"**; Manca F
CORPORATE SOURCE: GBF NATL RES CTR BIOTECHNOL,DIV MICROBIOL, MASCHERODER WEG 1/D-38124 BRAUNSCHWEIG//GERMANY/ (REPRINT); ADV BIOTECHNOL CTR,UNIT RETROVIRAL IMMUNOL/GENOA//ITALY/; GBF NATL RES CTR BIOTECHNOL,DIV CELL & IMMUNE BIOL/D-38124 BRAUNSCHWEIG//GERMANY/; UNIV GIESSEN KLINIKUM,INST MED MICROBIOL/D-6300 GIESSEN//GERMANY/
PUBLICATION TYPE: JOURNAL
PUBLICATION: EUROPEAN JOURNAL OF IMMUNOLOGY, 1998, V28, N6 (JUN), P 1807-1814
PUBLISHER: VCH PUBLISHERS INC, 303 NW 12TH AVE, DEERFIELD BEACH, FL 33442-1788
ISSN: 0014-2980
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: Listeria monocytogenes is a facultative intracellular pathogen which, following uptake by macrophages, escapes from the phagosome and replicates in the cytoplasm. This property has been exploited using recombinant L. monocytogenes as a carrier for the intracytoplasmic expression of antigens when MHC class I-restricted cytotoxic T lymphocyte responses are required. Much less is known of the ability of these bacteria to trigger MHC class II-restricted responses. Here, we demonstrate that after ingestion of L. monocytogenes expressing a T helper epitope from the gp120 envelope glycoprotein of HIV, human adherent macrophages and dendritic cells can process and present the epitope to a specific CD4(+) T cell line in the context of MHC class II molecules. No significant differences were observed when the attenuated strains were trapped in the phagolysosome or impaired in the capacity to spread intracellularly or from cell to cell. Similar results were obtained using carrier proteins that were either secreted, associated with the bacterial surface, or restricted to the bacterial cytoplasm. A dominant expression of the TCR V beta 22 gene subfamily was observed in specific T cell lines generated after stimulation with the recombinant strains or with soluble gp120. Our data show that in this in vitro

system L. monocytogenes can efficiently deliver antigens to the MHC class II pathway, in addition to the well-established MHC class I pathway. The eukaryotic cell compartment in which the antigen is synthesized, and the mode of display seem to play a minor role in the overall efficiency of epitope processing and presentation.

34/3,AB/9 (Item 5 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

08919417 GENUINE ARTICLE#: YC180 NUMBER OF REFERENCES: 58
TITLE: Crystal structure of the phosphatidylinositol-specific phospholipase C from the human pathogen Listeria monocytogenes
AUTHOR(S): Moser J; *Gerstel B"**; Meyer JEW; *Chakraborty T"**; Wehland J; Heinz DW (REPRINT)
CORPORATE SOURCE: UNIV FREIBURG,INST ORGAN CHEM & BIOCHEM, ALBERTSTR 21/D-79104 FREIBURG//GERMANY/ (REPRINT); UNIV FREIBURG,INST ORGAN CHEM & BIOCHEM/D-79104 FREIBURG//GERMANY/; GESELL BIOTECHNOL FORSCH MBH,/D-38124 BRAUNSCHWEIG//GERMANY/; UNIV GIESSEN,INST MED MIKROBIOL/D-35385 GIESSEN//GERMANY/
PUBLICATION TYPE: JOURNAL
PUBLICATION: JOURNAL OF MOLECULAR BIOLOGY, 1997, V273, N1 (OCT 17), P 269-282
PUBLISHER: ACADEMIC PRESS LTD, 24-28 OVAL RD, LONDON, ENGLAND NW1 7DX
ISSN: 0022-2836
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: The X-ray crystal structure of the phosphatidylinositol-specific phospholipase C (PT-PLC) from the human pathogen Listeria monocytogenes has been determined both in free form at 2.0 Angstrom resolution, and in complex with the competitive inhibitor myo-inositol at 2.6 Angstrom resolution. The structure was solved by a combination of molecular replacement using the structure of Bacillus cereus PI-PLC and single isomorphous replacement. The enzyme consists of a single (beta alpha)(8)-barrel domain with the active site located at the C-terminal side of the beta-barrel. Unlike other (beta alpha)(8)-barrels, the barrel in PI-PLC is open because it lacks hydrogen bonding interactions between beta-strands V and VI. myo-Inositol binds to the active site pocket by making specific hydrogen bonding interactions with a number of charged amino acid side-chains as well as a coplanar stacking interaction with a tyrosine residue. Despite a relatively low sequence identity of approximately 24%, the structure is highly homologous to that of B.cereus PI-PLC with an r.m.s. deviation for 228 common C-alpha positions of 1.46 Angstrom. Larger differences are found for loop regions that accommodate most of the numerous amino acid insertions and deletions. The active site pocket is also well conserved with only two amino acid replacements directly implicated in inositol binding. (C) 1997 Academic Press Limited.

34/3,AB/10 (Item 6 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

07324756 GENUINE ARTICLE#: UG238 NUMBER OF REFERENCES: 31
TITLE: APOPTOSIS OF MOUSE DENDRITIC CELLS IS TRIGGERED BY LISTERIOLYSIN, THE MAJOR VIRULENCE DETERMINANT OF LISTERIA MONOCYTOGENES
AUTHOR(S): GUZMAN CA; DOMANN E; ROHDE M; BRUDER D; DARJI A; *WEISS S"**;

WEHLAND J; *CHAKRABORTY T"**; *TIMMIS KN"**
CORPORATE SOURCE: GBF NATL RES CTR BIOTECHNOL,DIV MICROBIOL,MASCHERODER WEG 1/D-38124 BRAUNSCHWEIG//GERMANY/ (Reprint); GBF NATL RES CTR BIOTECHNOL,DIV CELL BIOL & IMMUNOL/D-38124 BRAUNSCHWEIG//GERMANY/; UNIV GIESSEN KLINIKUM,INST MED MIKROBIOL/D-35392 GIESSEN//GERMANY/
PUBLICATION: MOLECULAR MICROBIOLOGY, 1996, V20, N1 (APR), P119-126
ISSN: 0950-382X
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE
ABSTRACT: Infection of a murine-spleen dendritic cell line by Listeria monocytogenes was found to induce cell death through apoptosis. To characterize the bacterial product(s) involved in induction of apoptosis, dendritic cells were infected with the L. monocytogenes EGD strain and several isogenic mutants deficient in the production of individual listerial virulence factors. The ability to induce cellular apoptosis was retained by all mutants tested, except the prfA and Delta hly mutants, both of which are unable to produce listeriolysin. Apoptosis was also induced by purified listeriolysin suggesting that this protein directly induces apoptosis. Purified recombinant listeriolysins rendered either weakly haemolytic by a C-484 to S mutation, or nonhaemolytic by a W-491 to A mutation exhibited little or no capacity to induce apoptosis, indicating that both activities are associated within the same protein region. Treatment with purified lister iolysin or L. monocytogenes infection also triggers apoptosis in explanted bone-marrow dendritic cells. Thus invasion of dendritic cells by L. monocytogenes, which results in cell death, may play an important role in the pathogenesis of listerial infections by impairing immune responses, hindering bacterial clearance and promoting spread of the infection.

34/3,AB/11 (Item 7 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

06684809 GENUINE ARTICLE#: RQ792 NUMBER OF REFERENCES: 34
TITLE: INTERACTION OF LISTERIA MONOCYTOGENES WITH MOUSE DENDRITIC CELLS
AUTHOR(S): GUZMAN CA; ROHDE M; *CHAKRABORTY T"**; DOMANN E; HUDEL M; WEHLAND J; *TIMMIS KN"**
CORPORATE SOURCE: GBF NATL RES CTR BIOTECHNOL,DIV MICROBIOL,MASCHERODER WEG 1/D-38124 BRAUNSCHWEIG//GERMANY/ (Reprint); GBF NATL RES CTR BIOTECHNOL,DIV CELL BIOL & IMMUNOL/D-38124 BRAUNSCHWEIG//GERMANY/; UNIV GIESSEN KLINIKUM,INST MED MIKROBIOL/GIESSEN//GERMANY/
PUBLICATION: INFECTION AND IMMUNITY, 1995, V63, N9 (SEP), P3665-3673
ISSN: 0019-9567
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE
ABSTRACT: In this study, the interaction of murine dendritic cells with Listeria monocytogenes was investigated. Dendritic cells are efficient antigen-presenting cells, play a key role in the immune response, and are capable of migrating over substantial distances between sites of infection and lymphoid tissues. L. monocytogenes EGD invaded dendritic cells, escaped from phagosomes into the cytoplasm, and there directed actin nucleation, polymerization, and polarization in a typical fashion, thereby achieving intracellular movement and cell-to-cell spread, The internalization process appears to be independent of the inl locus, Interestingly, an intact microtubular function was essential for efficient uptake, whereas in a previous report, microtubule disruption did not affect bacterial spread in Caco-2 cells. The results obtained also suggest that L. monocytogenes binds to glycosylated

receptors of dendritic cells, Uptake of Listeria cells was mediated by a protein kinase-dependent transducing phosphorylation signal that induces the actin polymerization-dependent phagocytic process. To achieve efficient uptake, de novo protein synthesis of eukaryotic and prokaryotic cells is also required, Despite the killing of dendritic cells, wild-type bacteria were found to persist in small numbers in some cells for at least 24 h. When different isogenic mutants of the EGD strain were analyzed for their capability to interact with dendritic cells, it was observed that some virulence-attenuated mutants (i.e., prfA and Delta hly) persisted in large numbers for even longer times, Invasion of dendritic cells by L. monocytogenes, which in turn could result in either cell death or persistent infection, might have an important role in the pathogenesis of listeriosis, leading to impaired immune responses with inefficient bacterial clearance and/or promoting bacterial spread.

34/3,AB/12 (Item 8 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

06324998 GENUINE ARTICLE#: QU574 NUMBER OF REFERENCES: 48
TITLE: A FOCAL ADHESION FACTOR DIRECTLY LINKING INTRACELLULARLY MOTILE LISTERIA MONOCYTOGENES AND LISTERIA IVANOVII TO THE ACTIN-BASED CYTOSKELETON OF MAMMALIAN CELLS
AUTHOR(S): *CHAKRABORTY T"**; EBEL F; DOMANN E; NIEBUHR K; *GERSTEL B"**; PISTOR S; TEMMGROVE CJ; JOCKUSCH BM; REINHARD M; WALTERS U; WEHLAND J
CORPORATE SOURCE: INST MED MIKROBIOL,FRANKFURTER STR 107/D-35392 GIESSEN//GERMANY/ (Reprint); GESELL BIOTECHNOL FORSCH MBH,ZELLBIOL IMMUNBIOLABT/W-3300 BRAUNSCHWEIG//GERMANY/; INST ZOOL,ZELLBIOL ABT/D-38106 BRAUNSCHWEIG//GERMANY/; UNIV WURZBURG,MED KLIN/D-97080 WURZBURG//GERMANY/
PUBLICATION: EMBO JOURNAL, 1995, V14, N7 (APR 3), P1314-1321
ISSN: 0261-4189
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE
ABSTRACT: The surface-bound ActA polypeptide of the intracellular bacterial pathogen Listeria monocytogenes is the sole listerial factor needed for recruitment of host actin filaments by intracellularly motile bacteria. Here we report that following Listeria infection the host vasodilator-stimulated phosphoprotein (VASP), a microfilament- and focal adhesion-associated substrate of both the cAMP- and cGMP-dependent protein kinases, accumulates on the surface of intracytoplasmic bacteria prior to the detection of F-actin 'clouds'. VASP remains associated with the surface of highly motile bacteria, where it is polarly located, juxtaposed between one extremity of the bacterial surface and the front of the actin comet tail. Since actin filament polymerization occurs only at the very front of the tail, VASP exhibits properties of a host protein required to promote actin polymerization. Purified VASP binds directly to the ActA polypeptide in vitro. A ligand-overlay blot using purified radiolabelled VASP enabled us to identify the ActA homologue of the related intracellular motile pathogen, Listeria ivanovii, as a protein with a molecular mass of similar to 150 kDa. VASP also associates with actin filaments recruited by another intracellularly motile bacterial pathogen, Shigella flexneri. Hence, by the simple expedient of expressing surface-bound attractor molecules, bacterial pathogens effectively harness cytoskeletal components to achieve intracellular movement.

34/3,AB/13 (Item 1 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

01003514
ATTENUATED SALMONELLA STRAIN USED AS A VEHICLE FOR ORAL IMMUNIZATION
ABGESCHWACHTER SALMONELLENSTAMM, GEBRAUCHT ALS VEHIKEL ZUR ORALEN IMMUNISIERUNG
SOUCHE ATTENUEE DE SALMONELLA UTILISEE EN TANT QUE VEHICULE D'IMMUNISATION ORALE
PATENT ASSIGNEE:
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PATENT (CC, No, Kind, Date): EP 977874 A1 000209 (Basic)
WO 9848026 981029
APPLICATION (CC, No, Date): EP 97953786 971211; WO 97EP6933 971211
PRIORITY (CC, No, Date): EP 97106503 970418
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE
INTERNATIONAL PATENT CLASS: C12N-015/74; C07K-014/195; A61K-039/02; A61K-039/112
NOTE:
No A-document published by EPO
LANGUAGE (Publication,Procedural,Application): English; English; English
? log y
20mar02 11:07:48 User219783 Session D1806.5

forms of **ActA** and **listeriolysin** - two virulence factors of Listeria monocytogenes - that were controlled by an eukaryotic promoter have been used to transform a **S. typhimurium** aroA strain. Multiple or even single immunizations with these transformants induced a strong cytotoxic and helper T cell response as well as an excellent antibody response. Multiple immunizations with **listeriolysin** transformants protected the mice completely against a lethal challenge of L. monocytogenes. Partial protection was already obsd. with a single dose. **ActA** appeared not to be a protective antigen. The strength and the kinetics of the response suggested that the heterologous antigens were expressed within the eukaryotic host cells following transfer of **plasmid** DNA from the bacterial carrier strain. Transfer of **plasmid** DNA could be unequivocally shown in vitro using primary peritoneal macrophages. The demonstration of RNA splice products and expression of **.beta.-galactosidase** in the presence of tetracycline - an inhibitor of bacterial protein synthesis - indicated that the gene was expressed by host cells rather than bacteria. Oral genetic immunization with Salmonella carriers provides a highly versatile system for antigen delivery, represents a potent system to identify candidate protective antigens for vaccination, and will permit efficacious generation of antibodies against virtually any DNA segment encoding an open reading frame.

L19 ANSWER 15 OF 40 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 1999005344 MEDLINE
DOCUMENT NUMBER: 99005344 PubMed ID: 9787153
TITLE: Gene transfer in dendritic cells, induced by oral DNA vaccination with **Salmonella typhimurium**, results in protective immunity against a murine fibrosarcoma.
AUTHOR: Paglia P; Medina E; Arioli I; Guzman C A; Colombo M P
CORPORATE SOURCE: Division of Experimental Oncology D, Istituto Nazionale per Lo Studio e la Cura dei Tumori, Milano, Italy.
SOURCE: BLOOD, (1998 Nov 1) 92 (9) 3172-6.
Journal code: A8G; 7603509. ISSN: 0006-4971.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199811
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 19990115
Entered Medline: 19981130

AB A live attenuated AroA- auxotrophic mutant of **Salmonella typhimurium** (**SL7207**) has been used as carrier for the **pCMVbeta vector** that contains the **beta-galactosidase** (**beta-gal**) gene under the control of the immediate early promoter of Cytomegalovirus (**CMV**). We tested whether orally administered bacterial carrier could enter and deliver the transgene to antigen-presenting cells (APCs) through the natural enteric route of infection and whether **beta-gal** expression could generate a protective response against an aggressive murine fibrosarcoma transduced with the **beta-gal** gene

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(F1.A11) that behaves operationally as a tumor-associated antigen. After three courses, at 15-day intervals, mice developed both cell-mediated and systemic humoral responses to **beta-gal**. Mice vaccinated with the Salmonella harboring **pCMVbeta**, but not with **plasmid**-less carrier, showed resistance to a challenge with F1.A11 cells. These experiments suggest that Salmonella-based DNA immunization allows us to specifically target antigen expression in vivo to APCs. To prove that the transgene is actually expressed by APCs as a function of an eukaryotic promoter, the green fluorescent protein (GFP) was placed under the control of either the eukariotic **CMV** or a prokaryotic promoter. Using cytofluorometric analysis, GFP was detected only in splenocytes of mice receiving a Salmonella carrier harboring GFP under the **CMV** promoter. These results indicate that transgene expression occurs because of a Salmonella-mediated gene transfer to eukaryotic cells. Finally, approximately 19% of the splenocytes expressed GFP. Among them, F4/80(+) macrophages and CD11cbright dendritic cells (DCs) were scored as positive for GFP expression. Extensive work has been performed trying to optimize the way to transfect DCs, ex vivo, with genes coding for relevant antigens. We show here, for the first time, that DCs can be directly and specifically transduced in vivo such to induce DNA vaccination against tumors.

L19 ANSWER 16 OF 40 MEDLINE DUPLICATE 7

ACCESSION NUMBER: 1998136095 MEDLINE
DOCUMENT NUMBER: 98136095 PubMed ID: 9477228
TITLE: Metabolic activation of aromatic amine mutagens by simultaneous expression of human cytochrome P450 1A2, NADPH-cytochrome P450 reductase, and N-acetyltransferase in Escherichia coli.
AUTHOR: Josephy P D; Evans D H; Parikh A; Guengerich F P
CORPORATE SOURCE: Guelph-Waterloo Centre for Graduate Work in Chemistry, Department of Chemistry and Biochemistry, University of Guelph, Ontario, Canada.. josephy@chembio.uoguelph.ca
CONTRACT NUMBER: P30 ES00267 (NIEHS)
R35 CA44353 (NCI)
T32 GM07347 (NIGMS)
SOURCE: CHEMICAL RESEARCH IN TOXICOLOGY, (1998 Jan) 11 (1) 70-4.
Journal code: A5X; 8807448. ISSN: 0893-228X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199804
ENTRY DATE: Entered STN: 19980416
Last Updated on STN: 19980416
Entered Medline: 19980408

AB We describe the construction of a new strain of Escherichia coli designed to bioactivate aromatic amines and to detect their mutagenicity with high sensitivity. Strain DJ4309 bears two **plasmids**, a pACYC184-derived **plasmid** which expresses **Salmonella typhimurium** acetyl CoA:arylamine N-acetyltransferase (NAT) and a pBR322-derived **plasmid** which expresses human cytochrome P450 1A2 and

NADPH-cytochrome P450 reductase. The combined actions of these enzymes convert aromatic amines into reactive, mutagenic N-acetoxy esters. The strain also carries a mutated copy of the **lacZ** gene (on an F' factor) which reverts to the wild-type gene by a -(GpC) frameshift mutation. Strain DJ4309 expresses high levels of NAT and cytochrome P450 1A2 and is very sensitive to mutagenesis induced by representative aromatic amines. Mutagenicity of 2-aminoanthracene in strain DJ4309 is higher than can be obtained by rat liver homogenate 9000g supernatant (S9) activation in the parent strain lacking the P450 expression **vector**. Strain DJ4309 provides a useful system for detecting mutagenic aromatic amines and for studying their metabolism by human P450 1A2.

L19 ANSWER 17 OF 40 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 1998074797 MEDLINE
DOCUMENT NUMBER: 98074797 PubMed ID: 9413986
TITLE: Oral somatic transgene vaccination using attenuated **S. typhimurium**.
AUTHOR: Darji A; Guzman C A; Gerstel B; Wachholz P; Timmis K N; Wehland J; Chakraborty T; Weiss S
CORPORATE SOURCE: Division of Cell Biology and Immunology, Gesellschaft fur Biotechnologische Forschung, National Research Centre for Biotechnology, Braunschweig, Germany.
SOURCE: CELL, (1997 Dec 12) 91 (6) 765-75.
Journal code: CQ4; 0413066. ISSN: 0092-8674.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199801
ENTRY DATE: Entered STN: 19980129
Last Updated on STN: 19980129
Entered Medline: 19980113

AB An attenuated strain of **S. typhimurium** has been used as a vehicle for oral genetic immunization. Eukaryotic expression **vectors** containing truncated genes of **ActA** and **listeriolysin**--two virulence factors of Listeria monocytogenes--have been used to transform **S. typhimurium** aroA. Multiple or even single oral immunizations with such transformants induced excellent cellular and humoral responses. In addition, protective immunity was induced with **listeriolysin** transformants. The quality of the responses suggested a transfer of **plasmid** DNA from the bacterial carrier to the host. Such transfer was unequivocally shown in vitro with primary peritoneal macrophages. We describe a highly versatile system for antigen delivery, identification of protective antigens for vaccination, and efficient generation of antibodies against the product of open reading frames present on virtually any DNA segment.

L19 ANSWER 18 OF 40 MEDLINE
ACCESSION NUMBER: 1998020874 MEDLINE
DOCUMENT NUMBER: 98020874 PubMed ID: 9382730
TITLE: The Escherichia coli hemolysin secretion apparatus--a versatile antigen delivery system in attenuated Salmonella.
AUTHOR: Gentschev I; Dietrich G; Mollenkopf H J; Sokolovic Z; Hess J; Kaufmann S H; Goebel W
CORPORATE SOURCE: Theodor-Boveri-Institut fur Biowissenschaften

(Mikrobiologie), University of Wurzburg, Germany.
SOURCE: BEHRING INSTITUTE MITTEILUNGEN, (1997 Feb) (98) 103-13.
Journal code: 9KI; 0367532. ISSN: 0301-0457.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199711
ENTRY DATE: Entered STN: 19971224
Last Updated on STN: 19971224
Entered Medline: 19971110

AB The E. coli hemolysin (HlyA) secretion apparatus represents a type I secretion system that is fully functional in Salmonella. The system which consists of the two specific membrane proteins HlyB and HlyD and the outer membrane protein TolC, recognizes on HlyA a C-terminally located signal sequence of about 60 amino acids. Fusion proteins to which this signal sequence is covalently linked at the C-terminus are also recognized by this secretion apparatus. The efficiency of secretion is dependent on the rate of folding of the reporter protein. Secretion-competent regions of a given reporter protein that is not secretable as entire protein can be screened by a recently constructed transposon TnhlyAs which allows the insertion of the secretion signal into any region of the reporter protein. The genetic information for antigens of any source ranging in size between 10 and 1000 amino acids can be easily inserted into a recently constructed secretion **vector** which will allow the secretion of the fused antigen(s) in attenuated **Salmonella typhimurium** strains and in other attenuated Enterobacteriaceae. By manipulation of the **Hly** secretion system the antigen can be either completely secreted into the environment, fixed on the outer membrane or arrested in the cytoplasm of the used carrier strain. By the use of appropriate attenuated Salmonella strains the antigen is delivered in isolated compartments or to the cytosolic compartment. The extracellular delivery of such antigens is also possible with the help of appropriate carrier strains. The immunological consequences of the different display of the processed antigen will be discussed in the paper by Hess et al in this volume. With a similar antigen delivery system the easy identification and molecular characterization of unknown antigens recognized by the immune system in an infection is also feasible.

L19 ANSWER 19 OF 40 MEDLINE DUPLICATE 9

ACCESSION NUMBER: 96225903 MEDLINE
DOCUMENT NUMBER: 96225903 PubMed ID: 8625454
TITLE: A new **Salmonella typhimurium** NM5004 strain expressing rat glutathione S-transferase 5-5: use in detection of genotoxicity of dihaloalkanes using an SOS/umu test system.
AUTHOR: Oda Y; Yamazaki H; Thier R; Ketterer B; Guengerich F P; Shimada T
CORPORATE SOURCE: Osaka Prefectural Institute of Public Health, Japan.
CONTRACT NUMBER: CA44353 (NCI)
ES00267 (NIEHS)
SOURCE: CARCINOGENESIS, (1996 Feb) 17 (2) 297-302.
Journal code: C9T; 8008055. ISSN: 0143-3334.
PUB. COUNTRY: ENGLAND: United Kingdom

Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199606
ENTRY DATE: Entered STN: 19960708
Last Updated on STN: 19980206
Entered Medline: 19960624

AB The Escherichia coli mu operon was subcloned into a pKK233-2 **vector** containing rat glutathione S-transferase (GST) 5-5 cDNA and the **plasmid** thus obtained was introduced into **Salmonella typhimurium** TA1535. The newly developed strain **S.typhimurium** NM5004, was found to have 52-fold greater GST activity than the original umu strain **S.typhimurium** TA1535/pSK1002. We compared sensitivities of these two tester strains, NM5004 and TA1535/pSK1002, for induction of umuC gene expression with several dihaloalkanes which are activated or inactivated by GST 5-5 activity. The induction of umuC gene expression by these chemicals was monitored by measuring the cellular **beta-galactosidase** activity produced by umuC"**lacZ** fusion gene in these two tester strains. Ethylene dibromide, 1-bromo-2-chloroethane, 1,2-dichloroethane, and methylene dichloride induced umuC gene expression more strongly in the NM5004 strain than the original strain. 4-Nitroquinoline 1-oxide and N-methyl-N'-nitro-N-nitrosoguanidine were found to induce umuC gene expression to similar extents in both strains. In the case of 1-nitropyrene and 2-nitrofluorene, however, NM5004 strain showed weaker umuC gene expression responses than the original TA1535/pSK1002 strain. 1,2-Epoxy-3-(4'-nitrophenoxy)propane, a known substrate for GST 5-5, was found to inhibit umuC induction caused by 1-bromo-2-chloroethane. These results indicate that this new tester NM5004 strain expressing a mammalian GST theta class enzyme may be useful for studies of environmental chemicals proposed to be activated or inactivated by GST activity.

L19 ANSWER 20 OF 40 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1996:155232 BIOSIS
DOCUMENT NUMBER: PREV199698727367
TITLE: Conditionally replicative and conjugative **plasmids** carrying **lacZ**-alpha for cloning, mutagenesis, and allele replacement in bacteria.
AUTHOR(S): Metcalf, William W.; Jiang, Weihong; Daniels, Larry L.; Kim, Soo-Ki; Haldimann, Andreas; Wanner, Barry L. (1)
CORPORATE SOURCE: (1) Dep. Biol. Sci., Purdue Univ., W. Lafayette, IN 47907 USA
SOURCE: Plasmid, (1996) Vol. 35, No. 1, pp. 1-13. ISSN: 0147-619X.
DOCUMENT TYPE: Article
LANGUAGE: English

AB We describe several new cloning **vectors** for mutagenesis and allele replacement experiments. These **plasmids** have the R6K-gamma DNA replication origin (oriR-R6Kgamma) so they replicate only in bacteria supplying the II replication protein (encoded by pir), and they can be maintained at low or high **plasmid** copy number by using Escherichia coli strains encoding either wild-type or mutant forms of II. They also carry the RP4 transfer origin (oriT-RP4) so they can be transferred by

conjugation to a broad range of bacteria. Most of them encode **lacZ**-alpha for blue-white color screening of colonies for ones with **plasmids** carrying inserts, as well as the f1 DNA replication origin for preparation of single-stranded DNA. Particular **plasmids** are especially useful for allele replacement experiments because they also encode a positive counterselectable marker. One set carries terAR (from Tn10) that allows for positive selection of **plasmid**-free segregants as tetracycline-sensitive (Tet-S) recombinants. Another set carries sacB (from Bacillus subtilis) that allows selecting **plasmid** -free segregants as sucrose-resistant (Suc-R) ones. Accordingly, derivatives of these **plasmids** can be introduced into a non-pir host (via conjugative transfer, transformation, or electroporation), and integrants with the **plasmid** recombined into the chromosome via homologous sequences are selected using a **plasmid** antibiotic resistance marker. **Plasmid**-free segregants with an allele replacement can be subsequently selected as Tet-Ss or Suc-R recombinants. A number of additional features (including the presence of multiple cloning sites flanked by T3 and T7 RNA polymerase promoters) make these **plasmids** useful as general cloning **vectors** as well.

L19 ANSWER 21 OF 40 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 96099281 MEDLINE
DOCUMENT NUMBER: 96099281 PubMed ID: 8522504
TITLE: A system to generate chromosomal mutations in Lactococcus lactis which allows fast analysis of targeted genes.
AUTHOR: Law J; Buist G; Haandrikman A; Kok J; Venema G; Leenhouts K
CORPORATE SOURCE: Department of Genetics, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Haren, The Netherlands.
SOURCE: JOURNAL OF BACTERIOLOGY, (1995 Dec) 177 (24) 7011-8. Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U37409
ENTRY MONTH: 199601
ENTRY DATE: Entered STN: 19960219
Last Updated on STN: 19960219
Entered Medline: 19960122

AB A system for generating chromosomal insertions in lactococci is described. It is based on the conditional replication of lactococcal pWV01-derived Ori+ RepA- **vector** pORI19, containing **lacZ** alpha and the multiple cloning site of pUC19. Chromosomal AluI fragments of Lactococcus lactis were cloned in pORI19 in RepA+ helper strain Escherichia coli EC101. The frequency of Campbell-type recombinants, following introduction of this **plasmid** bank into L. lactis (RepA-), was increased by combining the system with temperature-sensitive pWV01 derivative pVE6007. Transformation of L. lactis MG1363 (pVE6007) with the pORI19 bank of lactococcal chromosomal fragments at the permissive temperature allowed replication of several copies of a recombinant **plasmid** from the bank within a cell because of the provision

in trans of RepA-Ts from pVE6007. A temperature shift to 37 degrees C resulted in loss of pVE6007 and integration of the pORI19 derivatives at high frequencies. A bank of lactococcal mutants was made in this way and successfully screened for the presence of two mutations: one in the monocistronic 1.3-kb peptidoglycan hydrolase gene (acmA) and one in the hitherto uncharacterized maltose fermentation pathway. Reintroduction of pVE6007 into the Mal- mutant at 30 degrees C resulted in excision of the integrated **plasmid** and restoration of the ability of ferment maltose. The integration **plasmid** (pMAL) was rescued by using the isolated **plasmid** content of a restored Mal+ colony to transform E. coli EC101. Nucleotide sequencing of the 564-bp chromosomal fragment in pMAL revealed an internal part of an open reading frame of which the translated product showed significant homology with ATP-binding proteins MalK of E. coli, **Salmonella typhimurium**, and Enterobacter aerogenes and MsmK of Streptococcus mutans. This combined use of two types of conditional replicating pWV01-derived **vectors** represents a novel, powerful tool for chromosomal gene inactivation, targeting, cloning, and sequencing of the labelled gene.

L19 ANSWER 22 OF 40 MEDLINE DUPLICATE 11
ACCESSION NUMBER: 95191566 MEDLINE
DOCUMENT NUMBER: 95191566 PubMed ID: 7885366
TITLE: Development of high sensitive umu test system: rapid detection of genotoxicity of promutagenic aromatic amines by **Salmonella typhimurium** strain NM2009 possessing high O-acetyltransferase activity.
AUTHOR: Oda Y; Yamazaki H; Watanabe M; Nohmi T; Shimada T
CORPORATE SOURCE: Osaka Prefectural Institute of Public Health, Japan.
SOURCE: MUTATION RESEARCH, (1995 Apr) 334 (2) 145-56.
Journal code: NNA; 0400763. ISSN: 0027-5107.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199504
ENTRY DATE: Entered STN: 19950425
Last Updated on STN: 19950425
Entered Medline: 19950412

AB A highly sensitive umu test system for the detection of carcinogenic/mutagenic aromatic amines has been developed utilizing a new tester strain, **Salmonella typhimurium** NM2009, possessing an elevated O-acetyltransferase (O-AT) level. NM2009 was constructed by subcloning the bacterial O-AT gene into a **plasmid vector** pACYC184 and introducing the **plasmid** into the original strain **S. typhimurium** TA1535/pSK1002 harboring an umuC'-'**lacZ** fusion gene. The system is based on the ability of DNA-damaging agents (genotoxins) to induce umuC gene expression and monitored by measuring the cellular **beta-galactosidase** activity evoked by the fusion gene. Twenty-two aromatic amine compounds including arylamines, aminoazo dyes, and heterocyclic aromatic amines were tested for inducibility of DNA damage after metabolic activation by rat liver S9 in strain NM2009 and the sensitivity was compared with those of the parent strain TA1535/pSK1002 and the O-AT-defective strain NM2000. NM2009 had

about 400 times higher O-AT activity than the parent strain. It was found that NM2009 was much more sensitive to aromatic amines than other strains to induce umuC gene expression after metabolic activation; the chemicals which were extremely sensitive in strain NM2009 include 2-aminoanthracene, 2-aminofluorene, 2-acetylaminofluorene, benzidine, 6-aminochrysene, 2,4-diaminotoluene, 2,6-diaminotoluene, 1-naphthylamine, o-tolidine, 3-MeO-AAB, o-aminoazotoluene, Glu-P-1, Trp-P-1, MeA alpha C, A alpha C, MeIQ, MeIQx, and IQ. In contrast, Trp-P-2 and PhIP showed almost similar sensitivities in three tester strains used in this study. These results suggest that strain NM2009 with high O-acetyltransferase activity is very useful to detect the genotoxic activities of potential mutagenic aromatic amine compounds, which require metabolic activation via the cytochrome P-450/acetyltransferase system.

L19 ANSWER 23 OF 40 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 93259169 MEDLINE
DOCUMENT NUMBER: 93259169 PubMed ID: 8491215
TITLE: Highly sensitive umu test system for the detection of mutagenic nitroarenes in **Salmonella typhimurium** NM3009 having high O-acetyltransferase and nitroreductase activities.
AUTHOR: Oda Y; Yamazaki H; Watanabe M; Nohmi T; Shimada T
CORPORATE SOURCE: Osaka Prefectural Institute of Public Health, Japan.
SOURCE: ENVIRONMENTAL AND MOLECULAR MUTAGENESIS, (1993) 21 (4) 357-64.
Journal code: EMM; 8800109. ISSN: 0893-6692.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199306
ENTRY DATE: Entered STN: 19930625
Last Updated on STN: 19930625
Entered Medline: 19930616

AB A highly sensitive umu test system for the detection of genotoxic activities of a variety of mutagenic nitroarenes has been developed using a new tester strain, **Salmonella typhimurium** NM3009 having high O-acetyltransferase (O-AT) and nitroreductase (NR) activities. The NM3009 was constructed by subcloning both the O-AT and NR genes into **plasmid vector** pACYC184, and the resulting **plasmid** was introduced into the parent tester strain **S. typhimurium** TA1535/pSK1002 harboring an umuC'-'**lacZ** fusion gene. The induction of umuC gene expression could be monitored by measuring the cellular **beta-galactosidase** activity produced by fusion gene. The purpose of the study was to evaluate whether the newly developed strain NM3009 is highly sensitive toward nitroarene compounds. The sensitivity of the strain NM3009 was compared with those of the parent TA1535/pSK1002 strain, the NR-overexpressing strain NM1011, the NR-deficient strain NM1000, the O-AT-overexpression strain NM2009, and the O-AT-defective strain NM2000. The newly developed NM3009 strain had about 13-fold and 3-fold higher activities for N-AT and NR, respectively, than the original **S. typhimurium** TA1535/pSK1002 strain. Among six strains tested, NM3009 showed the highest sensitivity toward such chemicals as 1-nitronaphthalene, 2-nitrofluorene,

3,7-dinitrofluoranthene, 3-nitrofluoranthene, 5-nitroacenaphthene, 2-nitronaphthalene, 1-nitropyrene, 1,6-dinitropyrene, 3,9-dinitrofluoranthene, 4,4'-dinitrobiphenyl, 1,8-dinitropyrene, m-dinitrobenzene, 2,4-dinitrotoluene, and 1,3-dinitropyrene. We have also found that the order of sensitivities to induce umuC gene expression toward a variety of nitroarenes was NM3009 > NM2009 > NM1011 > TA1535/pSK1002 > NM2000 > NM1000. These results suggest that the newly developed tester strain NM3009 is of great use for the detection of genotoxic activities of numerous carcinogenic and mutagenic chemicals including nitroarenes, which require NR and/or O-AT for the activation.

L19 ANSWER 24 OF 40 MEDLINE
ACCESSION NUMBER: 93175110 MEDLINE
DOCUMENT NUMBER: 93175110 PubMed ID: 8382417
TITLE: Stability, immunogenicity and expression of foreign antigens in bacterial vaccine **vectors**.
AUTHOR: Cardenas L; Clements J D
CORPORATE SOURCE: Department of Microbiology and Immunology, Tulane University School of Medicine, New Orleans, LA 70112.
CONTRACT NUMBER: AI28835 (NIAID)
SOURCE: VACCINE, (1993) 11 (2) 126-35.
Journal code: X6O; 8406899. ISSN: 0264-410X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199303
ENTRY DATE: Entered STN: 19930402
Last Updated on STN: 19990129
Entered Medline: 19930323

AB The use of attenuated strains of Salmonella as vaccine **vectors** frequently involves the introduction of heterologous antigens on recombinant **plasmids**. To overcome the problem of **plasmid** instability, we have integrated the gene that codes for a potential immunogen into the chromosome of a galE mutant of **Salmonella typhimurium**. Comparative in vitro and in vivo studies were conducted between the strain carrying the gene chromosomally integrated and an isogenic strain carrying the same gene on a multicopy **plasmid**. Levels of expression of the foreign antigen were significantly lower when the antigen was expressed from the chromosome than when it was expressed from the **plasmid**. The in vivo maintenance of the genes coding for antigen expression was determined on organisms recovered from spleen, liver and Peyer's patches of orally inoculated mice. By 24 h postinoculation, the majority of tissue isolates from the **plasmid**-containing strain had lost the **plasmid** and the ability to synthesize the antigen. By contrast, 100% of the recovered cointegrate isolates retained the ability to express the antigen throughout the 21 days of the experiment. Significantly, humoral and mucosal antibody levels against the antigen were greater when the antigen was expressed from the **plasmid** stabilized by the presence of the antibiotic than when the antigen was expressed from the chromosome. These observations indicate that the most important event for the development of an immune response against a foreign antigen delivered by these **vectors** may be the initial amount of antigen that primes the gut-associated lymphoid tissue and not persistence of the **vector** in

tissues.

L19 ANSWER 25 OF 40 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 94077065 MEDLINE
DOCUMENT NUMBER: 94077065 PubMed ID: 8255210
TITLE: Use of incompatible **plasmids** to control expression of antigen by **Salmonella typhimurium** and analysis of immunogenicity in mice.
AUTHOR: Ervin S E; Small P A Jr; Gulig P A
CORPORATE SOURCE: Department of Immunology and Medical Microbiology, University of Florida, College of Medicine, Gainesville 32610-0266.
CONTRACT NUMBER: AI-07713 (NIAID)
AI-28421 (NIAID)
SOURCE: MICROBIAL PATHOGENESIS, (1993 Aug) 15 (2) 93-101.
Journal code: MIC; 8606191. ISSN: 0882-4010.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199401
ENTRY DATE: Entered STN: 19940203
Last Updated on STN: 19940203
Entered Medline: 19940112

AB Salmonella spp. have been investigated as live vaccine **vectors** because they are heat stable and can elicit humoral, cellular, and secretory immune responses. However, the expression of some foreign antigens is toxic to bacterial **vectors**. We therefore studied an approach for the controlled expression of antigen in **Salmonella typhimurium** wherein the antigen is not expressed in vitro but is expressed in vivo. A model antigen, **beta-galactosidase**, was expressed from the trc promoter on one **plasmid**, while repression was achieved by LacI expressed in trans from a second **plasmid**. The second repressor **plasmid** was incompatible with the expression **plasmid** encoding **beta-galactosidase**. Loss by segregation of the repressor **plasmid** in vitro correlated with increased expression of **beta-galactosidase**. Oral inoculation of mice with salmonellae containing both **plasmids** induced serum IgG but not nasal, salivary, or biliary IgA antibody to **beta-galactosidase**. Serum IgG as well as biliary IgA anti-**S. typhimurium** antibody, but not salivary or nasal IgA, were also detected. This salmonella **vector** system for the controlled expression of recombinant antigens may be of value for inducing systemic but not mucosal immunity to antigens that are toxic to bacterial **vectors**.

L19 ANSWER 26 OF 40 EMBASE
ACCESSION NUMBER: 93000267 EMBASE
DOCUMENT NUMBER: 1993000267
TITLE: The putative sigma factor KatF (RpoS) is required for the transcription of the **Salmonella typhimurium** virulence gene spvB in Escherichia coli.
AUTHOR: Norel F.; Robbe-Saule V.; Popoff M.Y.; Coynault C.
CORPORATE SOURCE: Institut Pasteur, Unite des Enterobacteries, 28 Rue

du Docteur Roux,75724 Paris Cedex 15, France
SOURCE: FEMS Microbiology Letters, (1992) 99/2-3 (271-276).
ISSN: 0378-1097 CODEN: FMLED7
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The virulence of **Salmonella typhimurium** for mice is dependent on a **plasmid**-borne gene cluster termed spv. We previously determined that both **S. typhimurium** and Escherichia coli bacteria grown in a rich medium preferentially express the spv genes during the stationary phase of growth. In this study we evaluated the role of KatF, a putative sigma factor for starvation- and stationary phase-induced genes, in the expression of the spvB gene. The transcription of spvB in E. coli was compared in katF and wild-type backgrounds, using cloned spvB-**lacZ** and spvB-cat fusions. Expression of spvB was found to be greatly affected in katF mutants. Complementation experiments performed with the cloned katF gene confirmed that KatF is required for the expression of the **S. typhimurium** virulence gene spvB in E. coli.

L19 ANSWER 27 OF 40 MEDLINE DUPLICATE 14

ACCESSION NUMBER: 92041659 MEDLINE
DOCUMENT NUMBER: 92041659 PubMed ID: 1938945
TITLE: Multiple mechanisms contribute to osmotic inducibility of proU operon expression in Escherichia coli: demonstration of two osmoresponsive promoters and of a negative regulatory element within the first structural gene.
AUTHOR: Dattananda C S; Rajkumari K; Gowrishankar J
CORPORATE SOURCE: Centre for Cellular and Molecular Biology, Hyderabad, India.
SOURCE: JOURNAL OF BACTERIOLOGY, (1991 Dec) 173 (23) 7481-90.
Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199112
ENTRY DATE: Entered STN: 19920124
Last Updated on STN: 19920124
Entered Medline: 19911226

AB Transcription of the proU operon in Escherichia coli is induced several hundredfold upon growth of cells in media of elevated osmolarity. A low-copy-number promoter-cloning **plasmid vector**, with **lacZ** as the reporter gene, was used for assaying the osmoresponsive promoter activity of each of various lengths of proU DNA, generated by cloning of discrete restriction fragments and by an exonuclease III-mediated deletion approach. The results indicate that expression of proU in E. coli is directed from two promoters, one (P2) characterized earlier by other workers with the start site of transcription 60 nucleotides upstream of the initiation codon of the first structural gene (proV), and the other (P1) situated 250 nucleotides upstream of proV. Furthermore, a region of DNA within proV was shown to be involved in negative regulation of proU transcription; phage Mu dII1681-generated lac

fusions in the early region of proV also exhibited partial derepression of proU regulation, in comparison with fusions further downstream in the operon. Sequences around promoter P1, sequences around P2, and the promoter-downstream negative regulatory element, respectively, conferred approximately 5-, 8-, and 25-fold osmoresponsivity on proU expression. Within the region genetically defined to encode the negative regulatory element, there is a 116-nucleotide stretch that is absolutely conserved between the proU operons of E. coli and **Salmonella typhimurium** and has the capability of exhibiting alternative secondary structure. Insertion of this region of DNA into each of two different **plasmid vectors** was associated with a marked reduction in the mean topological linking number in **plasmid** molecules isolated from cultures grown in high-osmolarity medium. We propose that this region of DNA undergoes reversible transition to an underwound DNA conformation under high-osmolarity growth conditions and that this transition mediates its regulatory effect on proU expression.

L19 ANSWER 28 OF 40 MEDLINE DUPLICATE 15
ACCESSION NUMBER: 92041657 MEDLINE
DOCUMENT NUMBER: 92041657 PubMed ID: 1938944
TITLE: Characterization of the major promoter for the **plasmid**-encoded sucrose genes scrY, scrA, and scrB.
AUTHOR: Cowan P J; Nagesha H; Leonard L; Howard J L; Pittard A J
CORPORATE SOURCE: Department of Microbiology, University of Melbourne, Parkville, Victoria, Australia.
SOURCE: JOURNAL OF BACTERIOLOGY, (1991 Dec) 173 (23) 7464-70. Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M63038; GENBANK-M63255; GENBANK-M64328; GENBANK-M64329; GENBANK-M64330; GENBANK-M64331; GENBANK-M64332; GENBANK-M84013; GENBANK-S65448; GENBANK-S65502
ENTRY MONTH: 199112
ENTRY DATE: Entered STN: 19920124
Last Updated on STN: 19960129
Entered Medline: 19911226

AB Sucrose genes from a Salmonella thompson **plasmid** were cloned in Escherichia coli K-12. A physical map and a genetic map of the genes were constructed, revealing strong homology with the scr regulon from the **Salmonella typhimurium** **plasmid** pUR400. Two promoters were examined after being subcloned into transcriptional fusion **vectors**. Primer extension analysis and site-directed mutagenesis were used to identify the precise location of the promoter of scrY, scrA, and scrB. Transcription from this promoter was regulated over a 1,000-fold range by the combined effects of ScrR-mediated repression and catabolite repression. A putative cyclic AMP receptor protein binding site centered 72.5 bp upstream of the start point of transcription of scrY appeared to be essential for full activity of the scrY promoter. Transcription from the putative scrK promoter was far less sensitive to repression by ScrR. In ScrR+ cells,

readthrough transcription from the putative scrK promoter into scrY accounted for less than 10% of scrY expression.

L19 ANSWER 29 OF 40 MEDLINE DUPLICATE 16
ACCESSION NUMBER: 92041634 MEDLINE
DOCUMENT NUMBER: 92041634 PubMed ID: 1938926
TITLE: One-step cloning system for isolation of bacterial lexA-like genes.
AUTHOR: Calero S; Garriga X; Barbe J
CORPORATE SOURCE: Department of Genetics and Microbiology, Autonomous University of Barcelona, Bellaterra, Spain.
SOURCE: JOURNAL OF BACTERIOLOGY, (1991 Nov) 173 (22) 7345-50. Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199112
ENTRY DATE: Entered STN: 19920124
Last Updated on STN: 19920124
Entered Medline: 19911220

AB A system to isolate lexA-like genes of bacteria directly was developed. It is based upon the fact that the presence of a lexA(Def) mutation is lethal to SulA+ cells of Escherichia coli. This system is composed of a SulA- LexA(Def) HsdR- strain and a lexA-conditional killer **vector** (**plasmid** pUA165) carrying the wild-type sulA gene of E. coli and a polylinker in which foreign DNA may be inserted. By using this method, the lexA-like genes of **Salmonella typhimurium**, Erwinia carotovora, Pseudomonas aeruginosa, and P. putida were cloned. We also found that the LexA repressor of **S. typhimurium** presented the highest affinity for the SOS boxes of E. coli in vivo, whereas the LexA protein of P. aeruginosa had the lowest. Likewise, all of these LexA repressors were cleaved by the activated RecA protein of E. coli after DNA damage. Furthermore, under high-stringency conditions, the lexA gene of E. coli hybridized with the lexA genes of **S. typhimurium** and E. carotovora but not with those of P. aeruginosa and P. putida.

L19 ANSWER 30 OF 40 MEDLINE DUPLICATE 17
ACCESSION NUMBER: 92140047 MEDLINE
DOCUMENT NUMBER: 92140047 PubMed ID: 1779768
TITLE: Regulation of toxA and regA by the Escherichia coli fur gene and identification of a Fur homologue in Pseudomonas aeruginosa PA103 and PA01.
AUTHOR: Prince R W; Storey D G; Vasil A I; Vasil M L
CORPORATE SOURCE: Department of Microbiology and Immunology, University of Colorado Health Sciences Center, Denver 80262.
CONTRACT NUMBER: AI15940 (NIAID)
SOURCE: MOLECULAR MICROBIOLOGY, (1991 Nov) 5 (11) 2823-31. Journal code: MOM; 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199203
ENTRY DATE: Entered STN: 19920329
Last Updated on STN: 19970203

Entered Medline: 19920310

AB A multicopy **plasmid** containing the Escherichia coli fur gene was introduced into Pseudomonas aeruginosa strain PA103C. This strain contains a toxA-**lacZ** fusion integrated into its chromosome at the toxA locus. **Beta-galactosidase** synthesis in this strain is regulated by iron, as is seen for exotoxin A production. **Beta-galactosidase** synthesis and exotoxin A production in PA103C containing multiple copies of E. coli fur was still repressed in low iron conditions. The transcription of regA, a positive regulator of toxA, was also found to be inhibited by multiple copies of the E. coli fur gene. In addition, the ability of PA103C containing multiple copies of E. coli fur to produce protease was greatly reduced relative to PA103C containing a **vector** control. A polyclonal rabbit serum containing antibodies that recognize E. coli Fur was used to screen whole-cell extracts from Vibrio cholerae, Shigella flexneri, **Salmonella typhimurium** and Pseudomonas aeruginosa. All strains tested expressed a protein that was specifically recognized by the anti-Fur serum. These results and those described above suggest that Fur structure and function are conserved in a variety of distinct bacterial genera and that at least some of these different genera use this regulatory protein to control genes encoding virulence factors.

L19 ANSWER 31 OF 40 MEDLINE DUPLICATE 18

ACCESSION NUMBER: 91105065 MEDLINE
DOCUMENT NUMBER: 91105065 PubMed ID: 2271712
TITLE: Catalytic activities of human liver cytochrome P-450 IIIA4 expressed in Saccharomyces cerevisiae.
AUTHOR: Brian W R; Sari M A; Iwasaki M; Shimada T; Kaminsky L S; Guengerich F P
CORPORATE SOURCE: Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0146.
CONTRACT NUMBER: CA 44353 (NCI)
ES 00267 (NIEHS)
RR 01688 (NCRR)
+
SOURCE: BIOCHEMISTRY, (1990 Dec 25) 29 (51) 11280-92.
Journal code: A0G; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199102
ENTRY DATE: Entered STN: 19910329
Last Updated on STN: 19970203
Entered Medline: 19910227

AB A human liver cytochrome P-450 (P-450) IIIA4 cDNA clone was inserted behind an alcohol dehydrogenase promoter in the **plasmid** **vector** pAAH5 and expressed in Saccharomyces cerevisiae (D12 and AH22 strains). A cytochrome P-450 with typical spectral properties was expressed at a level of approximately 8 x 10(5) molecules/cell in either strain of yeast. The expressed P-450 IIIA4 had the same apparent monomeric Mr as the corresponding protein in human liver microsomes (P-450NF) and could be isolated from yeast microsomes. Catalytic activity of the yeast microsomes toward putative P-450 IIIA4 substrates was seen in the reactions supported by cumene hydroperoxide but was often lower and variable when

supported by the physiological donor NADPH. The catalytic activity of purified P-450 IIIA4 was also poor in some systems reconstituted with rabbit liver NADPH-P-450 reductase and best when both the detergent 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate and a lipid extract (from liver or yeast microsomes) or L-alpha-1,2-dilauroyl-sn-glycero-3-phosphocholine were present. Under these conditions the expressed P-450 IIIA4 was an efficient catalyst for nifedipine oxidation, 6 beta-hydroxylation of testosterone and cortisol, 2-hydroxylation of 17 beta-estradiol and 17 alpha-ethynylestradiol, N-oxygenation and 3-hydroxylation of quinidine, 16 alpha-hydroxylation of dehydroepiandrosterone 3-sulfate, erythromycin N-demethylation, the 10-hydroxylation of (R)-warfarin, the formation of 9,10-dehydrowarfarin from (S)-warfarin, and the activation of aflatoxins B1 and G1, sterigmatocystin, 7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene (both + and - diastereomers), 3,4-dihydroxy-3,4-dihydrobenz[a]anthracene, 3,4-dihydroxy-3,4-dihydro-7, 12-dimethylbenz[a]anthracene, 9,10-dihydroxy-9,10-dihydrobenzo[b]fluoranthene, 6-aminochrysene, and tris(2,3-dibromopropyl) phosphate to products genotoxic in a **Salmonella typhimurium** TA1535/pSK1002 system where a chimeric umuC' **'lacZ plasmid** is responsive to DNA alkylation. Reaction rates were stimulated by 7,8-benzoflavone and inhibited by rabbit anti-P-450 IIIA (anti-P-450NF), troleandomycin, gestodene, and cimetidine. Evidence was obtained that rates of reduction of ferric P-450 IIIA4 in yeast microsomes and the reconstituted systems are slow and at least partially responsible for the lower rates of catalysis seen in these systems (relative to liver microsomes). The results of these studies with a defined protein clearly demonstrate the ability of P-450 IIIA4 to catalyze regio- and stereoselective oxidations with a diverse group of substrates, and this enzyme appears to be one of the most versatile catalysts in the P-450 family.

L19 ANSWER 32 OF 40 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1991:112086 BIOSIS
DOCUMENT NUMBER: BA91:59476
TITLE: CATALYTIC ACTIVITIES OF HUMAN LIVER CYTOCHROME P-450 IIIA4 EXPRESSED IN SACCHAROMYCES-CEREVISIAE.
AUTHOR(S): BRIAN W R; SARI M-A; IWASAKI M; SHIMADA T; KAMINSKY L S; GUENGERICH F P.
CORPORATE SOURCE: DEP. BIOCHEM., VANDERBILT UNIV. SCH. MED., NASHVILLE, TENN. 37232-0146.
SOURCE: BIOCHEMISTRY, (1990) 29 (5), 11280-11292.
CODEN: BICHAW. ISSN: 0006-2960.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB A human liver cytochrome P-450 (P-450) IIIA4 cDNA clone was inserted behind an alcohol dehydrogenase promoter in the **plasmid vector** pAAH5 and expressed in Saccharomyces cerevisiae (D12 and AH22 strains). A cytochrome P-450 with typical spectral properties was expressed at a level of .apprx. 8 .times. 105 molecules/cell in either strain of yeast. The expressed P-450 IIIA4 had the same apparent monomeric Mr as the corresponding protein in human liver microsomes (P-450NF) and could be isolated from yeast microsomes. Catalytic activity of the yeast microsomes toward putative P-450 IIIA4 substrates was seen in the reactions supported by cumene hydroperoxide but was often lower and variable when supported by the physiological donor NADPH. The catalytic activity

of purified P-450 IIIA4 was also poor in some systems reconstituted with rabbit liver NADPH-P-450 reductase and best when both the detergent 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate and a lipid extract (from liver or yeast microsomes) or L-.alpha.-1,2-dilauroyl-sn-glycero-3-phosphocholine were present. Under these conditions the expressed P-450 IIIA4 was an efficient catalyst for nifedipine oxidation, 6.beta.-hydroxylation of testosterone and cortisol, 2-hdyroxylation of 17.beta.-estradiol and 17.alpha.-ethynylestradiol, N-oxygenation and 3-hydroxylation of quinidine, 16.alpha.-hydroxylation of dehydroepiandrosterone 3-sulfate, erythromycin N-demethylation, the 10-hydroxylation of (R)-warfarin, the formation of 9,10-dehydrowarfarin from (S)-warfarin, and the activation of aflatoxins B1 and G1, sterigmatocystin, 7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene (both + and - diastereomers), 3,4-dihydroxy-3,4-dihydrobenz[a]anthracene, 3,4-dihydroxy-3,4-dihydro-7,12-dimethylbenz[a]anthracene, 9,10-dihydroxy-9,10-dihydrobenzo[b]fluoranthene, 6-aminochrysene, and tris(2,3-dibromopropyl) phosphate to products genotoxic in a **Salmonella typhimurium** TA1535/pSK1002 system where a chimeric umuC' **'lacZ plasmid** is responsive to DNA alkylation. Reaction rates were stimulated by 7,8-benzoflavone and inhibited by rabbit anti-P-450 IIA (anti-P-450NF), troleandomycin, gestodene, and cimetidine. Evidence was obtained that rates of reduction of ferric P-450 IIIA4 in yeast microsomes and the reconstituted systems are slow and at least partially responsible for the lower rates of catalysis seen in these systems (relative to liver microsomes). The results of these studies with a defined protein clearly demonstrate the ability of P450 IIIA4 to catalyze regio- and stereoselective oxidations with a diverse group of substrates, and this enzyme appears to be one of the most versatile catalysts in the P-450 family.

L19 ANSWER 33 OF 40 MEDLINE DUPLICATE 19

ACCESSION NUMBER:	89359099 MEDLINE
DOCUMENT NUMBER:	89359099 PubMed ID: 2548994
TITLE:	Nucleotide sequence of the transcriptional control region of the osmotically regulated proU operon of **Salmonella typhimurium** and identification of the 5' endpoint of the proU mRNA.
AUTHOR:	Overdier D G; Olson E R; Erickson B D; Ederer M M; Csonka L N
CORPORATE SOURCE:	Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47906.
CONTRACT NUMBER:	1-RO1-GM 3194401 (NIGMS)
SOURCE:	JOURNAL OF BACTERIOLOGY, (1989 Sep) 171 (9) 4694-706. Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY:	United States
	Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:	English
FILE SEGMENT:	Priority Journals
OTHER SOURCE:	GENBANK-M26063
ENTRY MONTH:	198909
ENTRY DATE:	Entered STN: 19900309
	Last Updated on STN: 19990129
	Entered Medline: 19890927

AB Southern blot analysis of 15 proU transposon insertions in **Salmonella typhimurium** indicated that this operon is at least 3 kilobase pairs in length. The nucleotide sequence of

1.5-kilobase-pair fragment that contains the transcriptional control region of the proU operon and the coding sequences specifying 290 amino acids of the first structural gene of the operon was determined. The predicted amino acid sequence of the product of this gene shows extensive similarity to the HisP, MalK, and other proteins that are inner membrane-associated components of binding protein-dependent transport systems. S1 mapping and primer extension analysis of the proU mRNAs revealed several species with different 5' ends. Two of these endpoints are sufficiently close to sequences that have weak similarities to the consensus -35 and -10 promoter sequences that they are likely to define two transcription start sites. However, we cannot rule out the possibility that some or all of the 5' endpoints detected arose as a result of the degradation of a longer mRNA. The expression of proU-**lacZ** operon fusions located on **plasmids** was normal in **S. typhimurium** regardless of the **plasmid** copy number. The sequences mediating normal, osmoregulated expression of the proU operon were shown by subcloning to be contained on an 815-base-pair fragment. A 350-base-pair subclone of this fragment placed onto a **lacZ** expression **vector** directed a high-level constitutive expression of **beta-galactosidase**, suggesting that there is a site for negative regulation in the proU transcriptional control region which has been deleted in the construction of this **plasmid**.

L19 ANSWER 34 OF 40 MEDLINE DUPLICATE 20
ACCESSION NUMBER: 90066338 MEDLINE
DOCUMENT NUMBER: 90066338 PubMed ID: 2511419
TITLE: Molecular cloning and nucleotide sequencing of oxyR, the positive regulatory gene of a regulon for an adaptive response to oxidative stress in Escherichia coli: homologies between OxyR protein and a family of bacterial activator proteins.
AUTHOR: Tao K; Makino K; Yonei S; Nakata A; Shinagawa H
CORPORATE SOURCE: Laboratory of Radiation Biology, Faculty of Science, Kyoto University, Japan.
SOURCE: MOLECULAR AND GENERAL GENETICS, (1989 Sep) 218 (3) 371-6.
Journal code: NGP; 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199001
ENTRY DATE: Entered STN: 19900328
Last Updated on STN: 19900328
Entered Medline: 19900104

AB Treatment of Escherichia coli and **Salmonella typhimurium** cells with a low dose of hydrogen peroxide induces expression of a large number of genes, and confers resistance to oxidative stresses. The oxyR gene encodes a positive regulatory protein for a subset of these genes involved in the defense against oxidative damage. We cloned a DNA fragment that contains the E. coli oxyR region on a **plasmid vector**, and analyzed the nucleotide sequence of the gene. The amino acid sequence of OxyR protein, deduced from the nucleotide sequence, shows a high degree of homology to the sequences of a number of bacterial activator proteins including LysR, CysB, IlvY,

MetR and NodD. The product of the oxyR gene identified by the maxicell procedure was a 34 kDa protein, which agrees with the size predicted from the nucleotide sequence of the gene.

L19 ANSWER 35 OF 40 MEDLINE DUPLICATE 21
ACCESSION NUMBER: 89124888 MEDLINE
DOCUMENT NUMBER: 89124888 PubMed ID: 3065456
TITLE: Molecular cloning, physical mapping and expression of the bet genes governing the osmoregulatory choline-glycine betaine pathway of Escherichia coli.
AUTHOR: Andresen P A; Kaasen I; Styrvold O B; Boulnois G; Strom A R
CORPORATE SOURCE: Institute of Fisheries, University of Tromso, Norway.
SOURCE: JOURNAL OF GENERAL MICROBIOLOGY, (1988 Jun) 134 (Pt 6) 1737-46.
Journal code: I87; 0375371. ISSN: 0022-1287.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198903
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19900308
Entered Medline: 19890317

AB An analysis of the bet genes governing the osmoregulatory choline-glycine betaine pathway of Escherichia coli was performed. A 9 kb BamHI fragment, located 30 to 39 kb counterclockwise of the EcoRI site of **lacZ**, coded for all known Bet activities. The following genes were identified: the betA gene for the choline dehydrogenase, the betB gene for the betaine aldehyde dehydrogenase, and the betT gene or operon for the high-affinity choline transport. The betB and the betT genes were named in this paper, and the clockwise gene order was shown to be betA,B,T. Subcloning gave **plasmids** which expressed each of the three Bet activities separately. The cloned bet genes remained osmotically regulated, indicating the existence of several osmotically regulated promoters in the bet region. **Salmonella typhimurium**, which carried the bet region of E. coli in the broad-host-range **vector** pRK293 expressed the three Bet activities and displayed increased osmotic tolerance in the presence of choline.

L19 ANSWER 36 OF 40 MEDLINE
ACCESSION NUMBER: 89112167 MEDLINE
DOCUMENT NUMBER: 89112167 PubMed ID: 3146019
TITLE: The mglB sequence of **Salmonella typhimurium LT2**; promoter analysis by gene fusions and evidence for a divergently oriented gene coding for the mgl repressor.
AUTHOR: Benner-Luger D; Boos W
CORPORATE SOURCE: Department of Biology, University of Konstanz, Federal Republic of Germany.
SOURCE: MOLECULAR AND GENERAL GENETICS, (1988 Nov) 214 (3) 579-87.
Journal code: NGP; 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 198903
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19900308
Entered Medline: 19890306

AB The mglB gene of **Salmonella typhimurium LT2** coding for the galactose-binding protein (GBP) was sequenced. We compared the deduced amino acid sequence with the GBP sequence of Escherichia coli K 12. The mature proteins differ in only 19 of 309 amino acid residues, corresponding to 94% homology. Analysis of the mglB control region by promoter-probe **vectors** revealed that two promoters, P1 and P2, constitute the mgl control region (Pmgl). P1 and P2 function in a synergistic way. P1 is the main promoter of the operon; its activity is 20 times the activity of P2. Both promoters are activated by the cyclic adenosine monophosphate catabolite activator protein (cAMP/CAP) complex. While P1 is inactive in the absence of the cAMP/CAP complex, there is residual activity of P2 under these conditions. Studies on the inducibility of the mglBAEC operon using multicopy **plasmid** promoter-probe **vectors** were hampered by the titration of the mgl repressor resulting in a partially constitutive expression of the mgl operon. The results indicate that only P1 is responding to induction by D-fucose. A weak promoter, PD, within the P1 region but divergent to it was found. PD is neither stimulated by the cAMP/CAP complex nor by D-fucose. We cloned the gene located downstream to PD and found it to strongly repress the expression of the mgl operon. We termed this gene mglD. The presence of D-fucose abolished the repression caused by the **plasmid** -encoded mglD gene product.

L19 ANSWER 37 OF 40 MEDLINE DUPLICATE 22
ACCESSION NUMBER: 88121688 MEDLINE
DOCUMENT NUMBER: 88121688 PubMed ID: 2828886
TITLE: Use of lambda vehicles to isolate ompC-**lacZ** gene fusions in **Salmonella typhimurium LT2**.
AUTHOR: Harkki A; Karkku H; Palva E T
CORPORATE SOURCE: Department of Genetics, University of Helsinki, Finland.
SOURCE: MOLECULAR AND GENERAL GENETICS, (1987 Oct) 209 (3) 607-11.
Journal code: NGP; 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198803
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19900308
Entered Medline: 19880307

AB A novel **plasmid vector**, pAMH70 carrying both the lamB and nusA genes of Escherichia coli K12 was constructed. Introduction of this **plasmid** into **Salmonella typhimurium LT2** renders this bacterium both sensitive to lambda adsorption and able to sustain growth and lysogenization by lambda. Using this strain as a recipient, stable gene fusions to the gene encoding a major outer membrane porin protein OmpC, were constructed with a lambda vehicle lambda placMu. To confirm the actual site of fusions they were genetically mapped

and transducing phages carrying the ompC-**lacZ** fusion were isolated and relysogenized. The fusions were also shown to be to ompC by their regulatory properties.

L19 ANSWER 38 OF 40 MEDLINE DUPLICATE 23
ACCESSION NUMBER: 88167832 MEDLINE
DOCUMENT NUMBER: 88167832 PubMed ID: 2832253
TITLE: Cloning and characterization of the metC gene from **Salmonella typhimurium LT2**
.
AUTHOR: Park Y M; Stauffer G V
CORPORATE SOURCE: Department of Microbiology, University of Iowa, Iowa City 52242.
CONTRACT NUMBER: GM-26878 (NIGMS)
SOURCE: GENE, (1987) 60 (2-3) 291-7.
Journal code: FOP; 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198804
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19990129
Entered Medline: 19880428

AB The metC gene of **Salmonella typhimurium** was cloned into the **plasmid vectors** pACYC184 and pBR322. Genetic and biochemical experiments indicate that the region controlling metC gene expression is present on the cloned fragments. The location of the metC gene was determined by insertional inactivation with transposons Tn5 and mini-Mu. The gene product was identified in a minicell system as a 49-kDa polypeptide. The direction of transcription and translation was determined by correlating the orientation of mini-Mu insertions within the metC gene with the expression of the **lacZ** gene contained in mini-Mu.

L19 ANSWER 39 OF 40 MEDLINE DUPLICATE 24
ACCESSION NUMBER: 90027520 MEDLINE
DOCUMENT NUMBER: 90027520 PubMed ID: 2855830
TITLE: Expression of the SOS genes of Escherichia coli in **Salmonella typhimurium**.
AUTHOR: Barbe J; Vericat J A; Llagostera M; Guerrero R
CORPORATE SOURCE: Department of Microbiology, Autonomous University of Barcelona, Bellaterra, Spain.
SOURCE: MICROBIOLOGIA, (1985 Sep) 1 (1-2) 77-87.
Journal code: AIF; 8904895. ISSN: 0213-4101.
PUB. COUNTRY: Spain
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198912
ENTRY DATE: Entered STN: 19900328
Last Updated on STN: 19900328
Entered Medline: 19891204

AB To lysogenize **Salmonella typhimurium** by Lambda phage, a region of 10.2 kb of Escherichia coli DNA carrying the nusA gene was cloned in a **S. typhimurium** strain containing a F'112 **plasmid** which codifies for the lamB

region of E. coli. The strain of **S. typhimurium** obtained in this way, was lysogenized by lambda c IndO- bacteriophage harboring either a fusion between recA1 or sfiA genes of E. coli with **lacZ** gene. Likewise, pSE143 **plasmid** with a umu C::**lacZ** fusion was introduced in **S. typhimurium**. Afterwards, induction of these SOS genes was studied. Results obtained show that the basal transcription of both recA and sfiA genes of E. coli was higher in **S. typhimurium** than in E. coli. Nevertheless, induction of recA and sfiA genes by UV-irradiation and mitomycin C was higher in E. coli than in **S. typhimurium**. On the other hand, umuC gene of E. coli presents the same basal level of transcription in both E. coli and **S. typhimurium** species, although induction of this gene by UV-irradiation and mitomycin C was higher in **S. typhimurium** than in E. coli. Therefore, the **plasmid** pUA25 constructed in this work may be used to introduce, using the Lambda phage as a **vector**, the SOS genes of E. coli in other bacterial species which may be useful to study the relationship between their respective SOS systems.

L19 ANSWER 40 OF 40 MEDLINE DUPLICATE 25
ACCESSION NUMBER: 85234387 MEDLINE
DOCUMENT NUMBER: 85234387 PubMed ID: 3924896
TITLE: Characterization of the **Salmonella typhimurium** mgl operon and its gene products.
AUTHOR: Muller N; Heine H G; Boos W
SOURCE: JOURNAL OF BACTERIOLOGY, (1985 Jul) 163 (1) 37-45. Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198508
ENTRY DATE: Entered STN: 19900320
Last Updated on STN: 19900320
Entered Medline: 19850822

AB In **Salmonella typhimurium** and Escherichia coli the high-affinity galactose transport system, which contains a periplasmic galactose-binding protein as an essential component, is encoded by the mgl genes. The entire mgl region of **S. typhimurium** is contained on a 6.3-kilobase EcoRI restriction fragment, which has been cloned into **plasmid vectors**. We determined the extent of the mgl region on this fragment by Tn5 mutagenesis, examination of **lacZ** fusions to mgl genes, and subcloning smaller restriction fragments. Polyacrylamide gel electrophoresis of protein preparations derived from strains carrying different **plasmids** was used to identify the mgl gene products. We conclude that the mgl operon consists of four genes that form a single transcription unit: mglB, mglA, mglE, and mglC. The mglB gene codes for galactose-binding protein (33,000 daltons), mglA codes for a membrane-bound protein of 51,000 daltons, and mglC codes for a 29,000-dalton membrane protein. The mglE product was less well characterized. Its existence was inferred from a mglE-**lacZ** protein fusion located between mglA and mglC. In addition, the coupled transcription-translation in vitro system indicated that mglE codes for a 21,000-dalton protein.

(FILE 'CAPLUS, MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO, TOXCENTER, PHIC, PHIN' ENTERED AT 10:13:18 ON 20 MAR 2002)

- Author(s)

L20	179	SEA ABB=ON	PLU=ON	DARJI A?/AU
L21	942	SEA ABB=ON	PLU=ON	GUZMAN C?/AU
L22	1466	SEA ABB=ON	PLU=ON	TIMMIS K?/AU
L23	7357	SEA ABB=ON	PLU=ON	WEISS S?/AU
L24	55	SEA ABB=ON	PLU=ON	GERSTEL B?/AU
L25	1165	SEA ABB=ON	PLU=ON	CHAKRABORTY T?/AU
L26	32	SEA ABB=ON	PLU=ON	WACHHOLZ P?/AU
L27	8	SEA ABB=ON	PLU=ON	L20 AND L21 AND L22 AND L23 AND L24 AND L25 AND L26
L28	102	SEA ABB=ON	PLU=ON	L20 AND (L21 OR L22 OR L23 OR L24 OR L25 OR L26)
L29	129	SEA ABB=ON	PLU=ON	L21 AND (L22 OR L23 OR L24 OR L25 OR L26)
L30	27	SEA ABB=ON	PLU=ON	L22 AND (L23 OR L24 OR L25 OR L26)
L31	102	SEA ABB=ON	PLU=ON	L23 AND (L24 OR L25 OR L26)
L32	37	SEA ABB=ON	PLU=ON	L24 AND (L25 OR L26)
L33	11	SEA ABB=ON	PLU=ON	L25 AND L26
L34	36	SEA ABB=ON	PLU=ON	(L28 OR L29 OR L31 OR L20 OR L21 OR L22 OR L23 OR L24 OR L25 OR L26) AND L5
L35	88	SEA ABB=ON	PLU=ON	L27 OR L30 OR L32 OR L33 OR L34
L36	26	DUP REM L35 (62 DUPLICATES REMOVED)		

L36 ANSWER 1 OF 26 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 1

ACCESSION NUMBER: 2001:711690 CAPLUS

TITLE: Eukaryotic expression plasmid transfer from the intracellular bacterium Listeria monocytogenes to host cells

AUTHOR(S): Hense, Marc; Domann, Eugen; Krusch, Stefan; **Wachholz, Petra**; Dittmar, Kurt E. J.; Rohde, Manfred; Wehland, Jurgen; **Chakraborty, Trinad**; Weiss, Siegfried

CORPORATE SOURCE: Department of Cell Biology and Immunology, GBF, German Research Centre for Biotechnology, Braunschweig, D-38124, Germany

SOURCE: Cell. Microbiol. (2001), 3(9), 599-609
CODEN: CEMIF5; ISSN: 1462-5814

PUBLISHER: Blackwell Science Ltd.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The facultative intracellular, Gram-pos. bacterium Listeria monocytogenes invades phagocytic and non-phagocytic cells from the tissues and organs of a wide variety of animals and humans. Here, we report the use of these bacteria as vehicles for gene transfer. Eukaryotic expression plasmids were introduced into the nucleus of host cells following lysis of the intracytosolic, plasmid-carrying bacteria with antibiotics. Cell lines of different tissues and species could be transfected in this way. We examd. bacterial properties required for delivery of the expression plasmids and found that this was strictly dependent on the ability of these bacteria to both invade eukaryotic cells and egress from the vacuole into the cytosol of the infected host cells. Macrophagelike cell lines or primary, peritoneal macrophages proved to be almost refractory to Listeria-mediated gene transfer. Thus, attenuated L. monocytogenes represents a serious candidate for consideration as a DNA-transfer vehicle for in vivo somatic gene therapy. The potential for oral administration of L. monocytogenes and the ease

in producing and cultivating recombinant strains are further attributes that make its use as a gene transfer vehicle attractive.
REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L36 ANSWER 2 OF 26 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2001188454 EMBASE
TITLE: Bacteria-mediated transfer of eukaryotic expression plasmids into mammalian host cells.
AUTHOR: **Weiss S.**; Krusch S.
CORPORATE SOURCE: S. Weiss, German Res. Centre Biotechnology GBF, Molecular Immunology, Mascheroder Weg 1, D-38124 Braunschweig, Germany
SOURCE: Biological Chemistry, (2001) 382/4 (533-541). Refs: 45
ISSN: 1431-6730 CODEN: BICHF3
COUNTRY: Germany
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
037 Drug Literature Index
039 Pharmacy
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Invasive intracellular bacteria are able to transfer eukaryotic expression plasmids into mammalian host cells in vitro and in vivo. This can be used to induce immune responses toward protein antigens encoded by the plasmid or to complement genetic defects. Plasmid transfer takes place when the recombinant bacterium dies within the host cell, either due to metabolic attenuation or induction of autolysis. Alternatively, antibiotics can be used and spontaneous transfer has also been observed, indicating that this phenomenon might also occur under physiological conditions. Plasmid transfer has been reported for Shigella flexneri, **Salmonella typhimurium** and **S. typhi**, Listeria monocytogenes and recombinant Escherichia coli, but other invasive bacteria should also share this property. In vivo attempts were mainly directed toward vaccination using shigella and salmonella as carrier. So far a wide variety of antigens have been used succesfully in mice. Often this type of immunization was superior over direct application of antigen or using the same bacterium as a heterologous carrier expressing the antigen via a prokaryotic promoter. Characterization of the host cells revealed that macrophages and dendritic cells might be responsible for immune stimulation by either expressing the antigen or cross-presenting the antigen after uptake of apoptotic antigen expressing cells.

L36 ANSWER 3 OF 26 WPIDS COPYRIGHT 2002 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2000-256988 [22] WPIDS
DOC. NO. CPI: C2000-078557
TITLE: Attenuated gram-negative Salmonella cells, comprising inactivated genes in the SPI2 locus and useful for vaccinating against a range of disorders associated with microbial infections such as stomach and cervical cancers.
DERWENT CLASS: B04 D16

INVENTOR(S): APFEL, H; GUZM, N C A; HENSEL, M; HUECK, C; MEDINA, E; **GUZMAN, C A**
PATENT ASSIGNEE(S): (CREA-N) CREATOGEN BIOSCIENCES GMBH; (CREA-N) CREATOGEN AG
COUNTRY COUNT: 89
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000014240	A2	20000316	(200022)*	EN	147
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW					
AU 9958605	A	20000327	(200032)		
EP 1108034	A2	20010620	(200135)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					
BR 9914479	A	20010626	(200140)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000014240	A2	WO 1999-EP6514	19990903
AU 9958605	A	AU 1999-58605	19990903
EP 1108034	A2	EP 1999-946122	19990903
		WO 1999-EP6514	19990903
BR 9914479	A	BR 1999-14479	19990903
		WO 1999-EP6514	19990903

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9958605	A	Based on	WO 200014240
EP 1108034	A2	Based on	WO 200014240
BR 9914479	A	Based on	WO 200014240

PRIORITY APPLN. INFO: EP 1998-116827 19980904
AN 2000-256988 [22] WPIDS
AB WO 200014240 A UPAB: 20000508
NOVELTY - Attenuated gram-negative cells (HC1), especially Salmonella, in which at least 1 gene in the SPI2 locus has been inactivated resulting in attenuation/reduction of virulence compared to the wild type cell, are new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:

(1) an isolated nucleic acid molecule (NAM1) comprising at least 50 nucleotides:

(a) of 2 defined nucleic acid sequences ((I) and (II));

(b) of an allele of (I) and/or (II);or

(c) of a nucleic acid sequence which hybridizes under stringent conditions to (I) and/or (II);

(2) a recombinant vector (VEC1) comprising NAM1;

(3) a host cell (HC1) comprising either NAM1 or VEC1;

(4) a polypeptide (PEP1) comprising:
(a) one of 17 defined amino acid sequences ((XXI) - (XXXVII)) given in the specification; or
(b) a sequence 60% homologous to (XXI) - (XXXVII);
(5) an antibody directed against PEP1;
(6) a fusion protein (PEP2) comprisng PEP1, which has been inserted, deletion-inserted or fused C- or NH2-terminally with at least one heterologous peptide;
(7) a composition comprising HC1 and an adjuvant;
(8) a method (A) for producing a living vaccine (i.e. HC1), comprising providing a living gram negative cell comprising the SPI2 locus and inactivating at least 1 gene of the locus to obtain attenuated HC1 cells;
(9) a method for the detection of attenuated cells (i.e. HC1) comprising providing a sample containing the cell and detecting a property not present in the wild type cells;
(10) a method (B) for establishing a library of attenuated gram-negative cells for the presentation of an antigen to a host, comprising obtaining at least 2 attenuated gram-negative cells (i.e. HC1), determining the pathogenicities of the cells and determining the relation of those pathogenicities;
(11) the use of the SPI2 locus, NAM1 and VEC1 for the preparation of HC1 for the presentation of an antigen to a cell; and
(12) an isolated nucleic acid molecule (NAM2) comprising at least 100 nucleotides:
(a) of 2 defined nucleic acid sequences ((XXVIII) and (IXXX)); or
(b) of a nucleic acid sequence which hybridizes under stringent conditions to (XXVIII) and/or (IXXX).

ACTIVITY - Cytostatic; anti-arteriosclerotic; anti-Alzheimer's; virucide; hepatotropic; antiinflammatory; bactericide.

MECHANISM OF ACTION - Vaccine.

The presence of **beta -galactosidase** (**beta -gal**) (which acted as an antigen) specific antibodies in intestinal washes from mice immunized with MvP101 or MvP103 (sseC::aphT and sseD::aphT mutant **Salmonella typhimurium** strains) carrying pAH97 was investigated 52 days after immunization. It was found that both carriers stimulated the production of significant amounts of **beta -gal** -specific immunoglobulin (Ig) A and to a lesser extent, favored the transudation of antigen-specific IgG in the intestinal lumen. Immunization with MvP103/pAH97 resulted in 4% of the total Ig obtained from intestinal lavages being IgA specific for **beta -gal** and 0.25% of the Ig was IgB specific for **beta -gal**. Immunization with MvP101/pAH97 resulted in 4.25% of the total Ig obtained from intestinal lavages being IgA specific for **beta -gal** and 1% of the Ig was IgB specific for **beta -gal**. No significant differences were observed among the mucosal responses to the different recombinant clones.

USE - The attenuate cells are used as carriers for presenting bacterial, viral or tumor antigens to a host and are capable of expressing the nucleic acid molecules in a target cell, especially a macrophage (claimed). Therefore, the cells may be used for the preparation of a prophylactic or therapeutic composition for the treatment of a chronic disease caused by a bacterium or virus (claimed). Preferably, the disease is either a Salmonella infection or a tumor. The cells may therefore be used to vaccinate against a

range of bacterial and viral pathogens such as Helicobacter pylori (directly associated with stomach cancer), Chlamydia pneumoniae (associated with arteriosclerosis and Alzheimer's disease), Borrella burgdorferi, Nanobacteria (found in the chronically diseased kidneys of patients with crystalline deposits), Hepatitis virus (causative agent of Hepatitis B and C and associated with liver cancer), Human papilloma virus (HPV) (associated with cervical cancer) or Hepes virus (claimed). The nucleic acids may also be used for the detection of in vivo inducible promoters.
Dwg.0/20

L36 ANSWER 4 OF 26 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 2
ACCESSION NUMBER: 2000:748673 CAPLUS
DOCUMENT NUMBER: 134:67703
TITLE: Mutations of arginine residues within the 146-KKRRK-150 motif of the ActA protein of Listeria monocytogenes abolish intracellular motility by interfering with the recruitment of the Arp2/3 complex
AUTHOR(S): Pistor, Susanne; Grobe, Lothar; Sechi, Antonio S.; Domann, Eugen; **Gerstel, Birgit**; Machesky, Laura M.; **Chakraborty, Trinad**; Wehland, Jurgen
CORPORATE SOURCE: Department of Cell Biology, Gesellschaft fur Biotechnologische Forschung, Braunschweig, D-38124, Germany
SOURCE: Journal of Cell Science (2000), 113(18), 3277-3287
CODEN: JNCSAI; ISSN: 0021-9533
PUBLISHER: Company of Biologists Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The recruitment of actin to the surface of intracellular Listeria monocytogenes and subsequent tail formation is dependent on the expression of the bacterial surface protein ActA. Of the different functional domains of ActA identified thus far, the N-terminal region is absolutely required for actin filament recruitment and intracellular motility. Mutational anal. of this domain which abolished actin recruitment by intracellular Listeria monocytogenes identified two arginine residues within the 146-KKRRK-150 motif that are essential for its activity. More specifically, recruitment of the Arp2/3 complex to the bacterial surface, as assessed by immunofluorescence staining with antibodies raised against the p21-Arc protein, was not obtained in these mutants. Consistently, treatment of infected cells with latrunculin B, which abrogated actin filament formation, did not affect assocn. of ActA with p21-Arc at the bacterial surface. Thus, the initial recruitment of the Arp2/3 complex to the bacterial surface is independent of, and precedes, actin polymn. Our data suggest that binding of the Arp2/3 complex is mediated by specific interactions dependent on arginine residues within the 146-KKRRK-150 motif present in ActA.
REFERENCE COUNT: 47 THERE ARE 47 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L36 ANSWER 5 OF 26 MEDLINE
ACCESSION NUMBER: 2000203432 MEDLINE
DOCUMENT NUMBER: 20203432 PubMed ID: 10741391

TITLE: Modulation of host immune responses stimulated by Salmonella vaccine carrier strains by using different promoters to drive the expression of the recombinant antigen.

AUTHOR: Medina E; Paglia P; Rohde M; Colombo M P; **Guzman C A**

CORPORATE SOURCE: Department of Microbial Pathogenesis and Vaccine Research, GBF-National Research Centre for Biotechnology, Braunschweig, Germany.

SOURCE: EUROPEAN JOURNAL OF IMMUNOLOGY, (2000 Mar) 30 (3) 768-77.
Journal code: EN5; 1273201. ISSN: 0014-2980.

PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200004

ENTRY DATE: Entered STN: 20000427
Last Updated on STN: 20000427
Entered Medline: 20000419

AB We evaluated whether immune responses stimulated by Salmonella vaccine carriers can be modulated by using different promoters to drive antigen expression. Mice were orally immunized with strains transfected with plasmids carrying **beta-galactosidase** (**beta-gal**) under the control of either a constitutive or an in vivo-activated promoter. While alpha-gal-reactive IgG1, IgG2a, IgG2b and IgG3 were detected in sera of mice immunized with Salmonella expressing constitutively **beta-gal**, higher titers dominated by IgG2a and IgG2b were detected in sera when the in vivo-activated promoter was used. **beta-gal**-specific proliferative responses of spleen-derived CD4+ T lymphocytes were similar in both groups. However, CD4+ T lymphocytes from mice immunized with the constitutive promoter secreted IL-4, IL-5, IL-6, IL-10 and IFN-gamma (Th1/Th2 pattern), whereas CD4+ cells mainly secreted IFN-gamma (Th1 pattern) when the second construct was used. The spleens of all immunized mice contained **beta-gal**-reactive CD8+ CTL precursors. The vaccine prototypes were tested for their capacity to control seeding and/or development within the lung of an intravenously delivered aggressive fibrosarcoma transfected with **beta-gal**. Reduced metastasis and significantly increased mean survival times were observed in all vaccinated mice. However, protection was improved when the carrier expressed **beta-gal** upon infection (80 % versus 50% survival, $p < 0.05$).

L36 ANSWER 6 OF 26 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 3

ACCESSION NUMBER: 2000:189530 CAPLUS

DOCUMENT NUMBER: 133:3443

TITLE: Oral delivery of DNA vaccines using attenuated **Salmonella typhimurium** as carrier

AUTHOR(S): **Darji, A.**; zur Lage, S.; Garbe, A. I.; **Chakraborty, T.**; **Weiss, S.**

CORPORATE SOURCE: Molecular Immunology, GBF-National Research Center for Biotechnology, Braunschweig, D-38124, Germany

SOURCE: FEMS Immunol. Med. Microbiol. (2000), 27(4),

341-349
CODEN: FIMIEV; ISSN: 0928-8244
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The efficacious delivery of eukaryotic expression plasmids to inductive cells of the immune system constitutes a key prerequisite for the generation of effective DNA vaccines. Here, the authors have explored the use of bacteria as vehicles to orally deliver expression plasmids. Attenuated **S. typhimurium** aroA harboring eukaryotic expression plasmids that encoded virulence factors of Listeria monocytogenes were administered orally to BALB/c mice. Strong cytotoxic and helper T cell responses as well as antibody prodn. were elicited even after a single administration. Mice immunized 4 times with Salmonella that carried a eukaryotic expression plasmid encoding the secretory listerial protein **listeriolysin** were protected against a subsequent lethal challenge with this pathogen. A single dose was already partially protective. The efficiency of this vaccination procedure was due to transfer of the expression plasmid from the bacterial carrier to the mammalian host. Evidence for such an event could be obtained in vivo and in vitro. Expression of the desired antigen in various lymphoid tissues was already detectable 1 day after administration of the DNA vaccine and persisted for at least 1 mo in spleen and mesenteric lymph nodes. Induction of cytotoxic and helper T cell responses was obsd. in all mouse strains tested including outbred strains whereas antibodies were mainly detected in BALB/c. Furthermore, the authors could show that immunogenicity could be improved by increasing the invasiveness of the bacterial carrier.

REFERENCE COUNT: 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L36 ANSWER 7 OF 26 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 4

ACCESSION NUMBER: 1999:153547 CAPLUS
DOCUMENT NUMBER: 130:324097

TITLE: Pathogenicity island 2 mutants of **Salmonella typhimurium** are efficient carriers for heterologous antigens and enable modulation of immune responses

AUTHOR(S): Medina, Eva; Paglia, Paola; Nikolaus, Thomas; Muller, Astrid; Hensel, Michael; **Guzman, Carlos A.**
CORPORATE SOURCE: Department of Microbial Pathogenicity and Vaccine Research, Division of Microbiology, GBF-National Research Centre for Biotechnology, Braunschweig, D-38124, Germany

SOURCE: Infect. Immun. (1999), 67(3), 1093-1099
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The potential use as vaccine delivery system of **Salmonella typhimurium** strains harboring defined mutations in the sseC (HH104) and sseD (MvP101) genes, which encode putative effector proteins of the type III secretion system of Salmonella pathogenicity island 2, was evaluated and compared with that of the well-characterized aroA mutant strain **SL7207** by using .

beta.-galactosidase (**.beta.-Gal**
) as a model antigen. When orally administered to immune-competent or gamma interferon-deficient (IFN-.gamma.-/-) BALB/c mice, both mutants were highly attenuated (50% LD, > 109 bacteria). Both strains were also able to efficiently colonize and persist in Peyer's patches. Immunization with HH104 and MvP101 triggered . **beta.-Gal**-specific serum and mucosal antibody responses equiv. to or stronger than those obsd. in **SL7207** -immunized mice. Although IgG2 serum antibodies were dominant in all groups, IgG1 was also significantly increased in mice vaccinated with MvP101 and **SL7207**. Comparable **.beta.-Gal**-specific IgA and IgG antibodies were detected in intestinal lavages from mice immunized with the different strains. Antigen-specific CD4+ T-helper cells were generated after vaccination with all vaccine prototypes; however, responses were significantly more efficient when HH104 and MvP101 were used ($P < 0.05$). Significantly higher levels of IFN-.gamma. were produced by restimulated spleen cells from mice immunized with HH104 than from those vaccinated with the MvP101 or **SL7207** derivs. (P .ltoreq. 0.05). Interestingly, the three strains induced major histocompatibility complex class I-restricted CD8+ cytotoxic T cells against **.beta.-Gal**; however, cytotoxic T-lymphocyte responses were significantly stronger after immunization with HH104 ($P < 0.05$). These novel **S. typhimurium** attenuated strains constitute promising delivery systems for vaccine antigens. The qual. differences obsd. in the obtained responses with different carriers may be useful for those applications in which a targeted immunomodulation is required.

REFERENCE COUNT: 45 THERE ARE 45 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L36 ANSWER 8 OF 26 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 5

ACCESSION NUMBER: 1999:142521 CAPLUS
DOCUMENT NUMBER: 130:280533
TITLE: Salmonella vaccine carrier strains. Effective delivery system to trigger antitumor immunity by oral route
AUTHOR(S): Medina, Eva; **Guzman, Carlos A.**; Staendner, Lothar H.; Colombo, Mario P.; Paglia, Paola
CORPORATE SOURCE: Division Microbiology, National Research Center Biotechnology, GBF, Braunschweig, D-38124, Germany
SOURCE: Eur. J. Immunol. (1999), 29(2), 693-699
CODEN: EJIMAF; ISSN: 0014-2980
PUBLISHER: Wiley-VCH Verlag GmbH
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Recombinant Salmonella strains expressing heterologous antigens can be delivered by oral route triggering the elicitation of efficient antigen-specific humoral, T helper, and cytotoxic responses. The potential of attenuated Salmonella to trigger anti-tumor immunity was evaluated for the 1st time by **.beta.-galactosidase** (**.beta.-gal**) as a model tumor-assocd. antigen (TAA). **.beta.-Gal** was expressed in a **S. typhimurium** aroA vaccine carrier strain either constitutively or under the control of a

promoter activated upon infection. Oral immunization with both vaccine prototypes resulted in the elicitation of .**beta.**-**gal**-specific humoral and cell-mediated immunity. Although both strains were able to trigger antigen-specific CTL, responses were more efficient when the expression was controlled by the promoter activated upon infection. The anti-tumor efficacy of the stimulated response was validated by challenging vaccinated animals with an aggressive fibrosarcoma transfected with .**beta.**-**gal**, which operationally acts as a TAA. Both groups of vaccinated mice exhibited a redn. in tumor take and growth with respect to animals vaccinated with plasmidless carrier. The overall efficiency was better in the group in which .**beta.**-**gal** was controlled by the in vivo-activated promoter (85% vs. 54%).

L36 ANSWER 9 OF 26 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 6
ACCESSION NUMBER: 1998:709195 CAPLUS
DOCUMENT NUMBER: 129:329695
TITLE: An attenuated strain of **Salmonella typhimurium** carrying eukaryotic expression constructs for use in oral vaccines
INVENTOR(S): **Darji, Ayub; Guzman, Carlos; Timmis, Kenneth**; Wehland, Jurgen; **Weiss, Siegfried; Gerstel, Birgit; Chakraborty, Trinad; Wachholz, Petra**
PATENT ASSIGNEE(S): Gesellschaft Fur Biotechnologische Forschung m.b.H., Germany
SOURCE: PCT Int. Appl., 52 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9848026	A1	19981029	WO 1997-EP6933	19971211
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9857562	A1	19981113	AU 1998-57562	19971211
EP 977874	A1	20000209	EP 1997-953786	19971211
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRIORITY APPLN. INFO.: EP 1997-106503 19970418
WO 1997-EP6933 19971211

AB An attenuated strain of **Salmonella typhimurium** has been developed for use as a vehicle for oral genetic immunization. Eukaryotic expression vectors contg. the genes for .**beta.**-**galactosidase**, or truncated forms of **ActA** and **listeriolysin** - two virulence factors of Listeria monocytogenes - that were controlled by an eukaryotic

promoter have been used to transform a **S. typhimurium** aroA strain. Multiple or even single immunizations with these transformants induced a strong cytotoxic and helper T cell response as well as an excellent antibody response. Multiple immunizations with **listeriolysin** transformants protected the mice completely against a lethal challenge of L. monocytogenes. Partial protection was already obsd. with a single dose. **ActA** appeared not to be a protective antigen. The strength and the kinetics of the response suggested that the heterologous antigens were expressed within the eukaryotic host cells following transfer of plasmid DNA from the bacterial carrier strain. Transfer of plasmid DNA could be unequivocally shown in vitro using primary peritoneal macrophages. The demonstration of RNA splice products and expression of **.beta .-galactosidase** in the presence of tetracycline - an inhibitor of bacterial protein synthesis - indicated that the gene was expressed by host cells rather than bacteria. Oral genetic immunization with Salmonella carriers provides a highly versatile system for antigen delivery, represents a potent system to identify candidate protective antigens for vaccination, and will permit efficacious generation of antibodies against virtually any DNA segment encoding an open reading frame.

L36 ANSWER 10 OF 26 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 7
ACCESSION NUMBER: 1998:714930 CAPLUS
DOCUMENT NUMBER: 130:80092
TITLE: Gene transfer in dendritic cells, induced by oral DNA vaccination with **Salmonella typhimurium**, results in protective immunity against a murine fibrosarcoma
AUTHOR(S): Paglia, Paola; Medina, Eva; Arioli, Ivano; **Guzman, Carlos A.**; Colombo, Mario P.
CORPORATE SOURCE: Division of Experimental Oncology D, Istituto Nazionale per Lo Studio e la Cura dei Tumori, Milan, I-20133, Italy
SOURCE: Blood (1998), 92(9), 3172-3176
CODEN: BLOOAW; ISSN: 0006-4971
PUBLISHER: W. B. Saunders Co.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A live attenuated AroA- auxotrophic mutant of **Salmonella typhimurium** (**SL7207**) has been used as carrier for the pCMV.beta. vector that contains the **.beta.-galactosidase** (**.beta.-gal**) gene under the control of the immediate early promoter of Cytomegalovirus (CMV). We tested whether orally administered bacterial carrier could enter and deliver the transgene to antigen-presenting cells (APCs) through the natural enteric route of infection and whether **.beta.-gal** expression could generate a protective response against an aggressive murine fibrosarcoma transduced with the **.beta.-gal** gene (F1.A11) that behaves operationally as a tumor-assocd. antigen. After three courses, at 15-day intervals, mice developed both cell-mediated and systemic humoral responses to **.beta.-gal**. Mice vaccinated with the Salmonella harboring pCMV.beta., but not with plasmid-less carrier, showed resistance to a challenge with F1.A11 cells. These expts. suggest that Salmonella-based DNA immunization allows us to specifically target antigen expression in vivo to APCs.

To prove that the transgene is actually expressed by APCs as a function of an eukaryotic promoter, the green fluorescent protein (GFP) was placed under the control of either the eukaryotic CMV or a prokaryotic promoter. Using cytofluorometric anal., GFP was detected only in splenocytes of mice receiving a Salmonella carrier harboring GFP under the CMV promoter. These results indicate that transgene expression occurs because of a Salmonella-mediated gene transfer to eukaryotic cells. Finally, approx. 19% of the splenocytes expressed GFP. Among them, F4/80+ macrophages and CD11cbright dendritic cells (DCs) were scored as pos. for GFP expression. Extensive work has been performed trying to optimize the way to transfect DCs, ex vivo, with genes coding for relevant antigens. We show here, for the first time, that DCs can be directly and specifically transduced in vivo such to induce DNA vaccination against tumors.

REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L36 ANSWER 11 OF 26 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 8
ACCESSION NUMBER: 1998:572457 CAPLUS
DOCUMENT NUMBER: 129:274673
TITLE: The role of the bacterial membrane protein ActA in immunity and protection against Listeria monocytogenes
AUTHOR(S): Darji, Ayub; Bruder, Dunja; Zur Lage, Susanne; **Gerstel, Birgit; Chakraborty, Trinad**; Wehland, Jurgen; Weiss, Siegfried
CORPORATE SOURCE: Division of Cell Biology and Immunobiology, Gesellschaft fur Biotechnologische Forschung-National Research Center for Biotechnology, Braunschweig, Germany
SOURCE: J. Immunol. (1998), 161(5), 2414-2420
CODEN: JOIMA3; ISSN: 0022-1767
PUBLISHER: American Association of Immunologists
DOCUMENT TYPE: Journal
LANGUAGE: English
AB ActA, an essential virulence factor of Listeria monocytogenes, is an integral membrane protein that is required for intracellular motility, cell-to-cell spread, and rapid dissemination of the bacteria in the infected host. To reveal cytotoxic T cell responses against ActA we introduced a recombinant sol. form of ActA into the MHC class I-processing compartment of APC using a variant of listeriolysin mutated within its immunodominant MHC class I epitope. With this exptl. system we demonstrate that T cells are induced against ActA during a sublethal infection with L. monocytogenes. However, adoptively transferred cytotoxic CD8+ T cells specific for ActA did not protect mice against a subsequent challenge with this pathogen. This was due to an inability of APC to present ActA by either MHC class I or class II mols. as long as ActA remained tethered to the surface of intracellular viable bacteria. ActA was only presented when L. monocytogenes were engineered to secrete ActA or when the bacteria were killed by antibiotics during the assay. These findings raise questions on the general use of membrane proteins of pathogens as candidates for subunit vaccines.

L36 ANSWER 12 OF 26 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 9
ACCESSION NUMBER: 1998:373130 CAPLUS

DOCUMENT NUMBER: 129:50390
TITLE: Attenuated Listeria monocytogenes carrier strains can deliver an HIV-1 gp120 T helper epitope to MHC class II-restricted human CD4+ T cells
AUTHOR(S): Guzman, Carlos A.; Saverino, Daniele; Medina, Eva; Fenoglio, Daniela; **Gerstel, Birgit**; Merlo, Andrea; Li Pira, Giuseppina; Buffa, Francesca; **Chakraborty, Trinad**; Manca, Fabrizio
CORPORATE SOURCE: Division Microbiology, GBF-National Research Center Biotechnology, Braunschweig, D-38124, Germany
SOURCE: Eur. J. Immunol. (1998), 28(6), 1807-1814
CODEN: EJIMAF; ISSN: 0014-2980
PUBLISHER: Wiley-VCH Verlag GmbH
DOCUMENT TYPE: Journal
LANGUAGE: English

AB L. monocytogenes is a facultative intracellular pathogen which, following uptake by macrophages, escapes from the phagosome and replicates in the cytoplasm. This property was exploited using recombinant L. monocytogenes as a carrier for the intracytoplasmic expression of antigens when MHC class I-restricted cytotoxic T lymphocyte responses are required. Much less is known of the ability of these bacteria to trigger MHC class II-restricted responses. The authors demonstrate that after ingestion of L. monocytogenes expressing a T helper epitope from the gp120 envelope glycoprotein of HIV, human adherent macrophages, and dendritic cells can process and present the epitope to a specific CD4+ T cell line in the context of MHC class II mols. No differences were obsd. when the attenuated strains were trapped in the phagolysosome or impaired in the capacity to spread intracellularly or from cell to cell. Similar results were obtained using carrier proteins that were either secreted, assocd. with the bacterial surface, or restricted to the bacterial cytoplasm. A dominant expression of the TCR V.beta. 22 gene subfamily was obsd. in specific T cell lines generated after stimulation with the recombinant strains or with sol. gp120. These data show that in this in vitro system L. monocytogenes can efficiently deliver antigens to the MHC class II pathway, in addn. to the well-established MHC class I pathway. The eukaryotic cell compartment in which the antigen is synthesized, and the mode of display seem to play a minor role in the overall efficiency of epitope processing and presentation.

L36 ANSWER 13 OF 26 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1998:384285 BIOSIS
DOCUMENT NUMBER: PREV199800384285
TITLE: Functional analysis of the N-terminal region of the ActA protein from Listeria monocytogenes.
AUTHOR(S): Pistor, Susanne (1); **Gerstel, Birgit (1)**; Groebe, Lothar (1); Domann, Eugen; **Chakraborty, Trinad**; Wehland, Juergen
CORPORATE SOURCE: (1) Abt. Zellbiol., GBF, Mascheroder Weg. 1, D-38124 Braunschweig Germany
SOURCE: European Journal of Cell Biology, (1998) Vol. 75, No. SUPPL. 48, pp. 63.
Meeting Info.: 22nd Annual Meeting of the Deutsche Gesellschaft fuer Zellbiologie (German Society for

Cell Biology) Saarbruecken, Germany March 15-19, 1998
German Society for Cell Biology
. ISSN: 0171-9335.
DOCUMENT TYPE: Conference
LANGUAGE: English

L36 ANSWER 14 OF 26 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 10
ACCESSION NUMBER: 1997:809173 CAPLUS
DOCUMENT NUMBER: 128:113825
TITLE: Oral somatic transgene vaccination using attenuated **S. typhimurium**
AUTHOR(S): **Darji, Ayub; Guzman, Carlos A.** ; **Gerstel, Birgit; Wachholz, Petra; Timmis, Kenneth N.**; Wehland, Jurgen; **Chakraborty, Trinad; Weiss, Siegfried**
CORPORATE SOURCE: Division of Cell Biology and Immunology, Gesellschaft fur Biotechnologische Forschung, National Research Centre for Biotechnology, Braunschweig, D-38124, Germany
SOURCE: Cell (Cambridge, Mass.) (1997), 91(6), 765-775
CODEN: CELLB5; ISSN: 0092-8674
PUBLISHER: Cell Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB An attenuated strain of **S. typhimurium** has been used as a vehicle for oral genetic immunization. Eukaryotic expression vectors contg. truncated genes of **ActA** and **listeriolysin**-two virulence factors of Listeria monocytogenes-have been used to transform **S. typhimurium** aroA. Multiple or even single oral immunizations with such transformants induced excellent cellular and humoral responses. In addn., protective immunity was induced with **listeriolysin** transformants. The quality of the responses suggested a transfer of plasmid DNA from the bacterial carrier to the host. Such transfer was unequivocally shown in vitro with primary peritoneal macrophages. We describe a highly versatile system for antigen delivery, identification of protective antigens for vaccination, and efficient generation of antibodies against the product of open reading frames present on virtually any DNA segment.

L36 ANSWER 15 OF 26 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 11
ACCESSION NUMBER: 1997:698571 CAPLUS
DOCUMENT NUMBER: 127:289849
TITLE: Crystal structure of the phosphatidylinositol-specific phospholipase C from the human pathogen Listeria monocytogenes
AUTHOR(S): Moser, Jurgen; **Gerstel, Birgit**; Meyer, Joachim E. W.; **Chakraborty, Trinad**; Wehland, Jurgen; Heinz, Dirk W.
CORPORATE SOURCE: Institut fur Organische Chemie und Biochemie, Universitat Freiburg, Freiburg, D-79104, Germany
SOURCE: J. Mol. Biol. (1997), 273(1), 269-282
CODEN: JMOBAK; ISSN: 0022-2836
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The x-ray crystal structure of the phosphatidylinositol-specific

phospholipase C (PI-PLC) from L. monocytogenes was detd. both in free form at 2.0 .ANG. resoln., and in complex with the competitive inhibitor, myo-inositol, at 2.6 .ANG. resoln. The structure was solved by a combination of mol. replacement using the structure of Bacillus cereus PI-PLC and single isomorphous replacement. The enzyme consisted of a single (.beta..alpha.)8-barrel domain with the active site located at the C-terminal side of the .beta.-barrel. Unlike other (.beta..alpha.)8-barrels, the barrel in PI-PLC was open because it lacked H-bonding interactions between .beta.-strands V and VI. Myo-Inositol bound to the active site pocket by making specific H-bonding interactions with a no. of charged amino acid side-chains as well as a coplanar stacking interaction with a Tyr residue. Despite a relatively low sequence identity of .apprx.24%, the structure was highly homologous to that of B.cereus PI-PLC with an r.m.s. deviation for 228 common C.alpha. positions of 1.46 .ANG.. Larger differences were found for loop regions that accommodated most of the numerous amino acid insertions and deletions. The active site pocket was also well conserved with only 2 amino acid replacements directly implicated in inositol binding.

L36 ANSWER 16 OF 26 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 12
ACCESSION NUMBER: 1996:327956 CAPLUS
DOCUMENT NUMBER: 125:51738
TITLE: The ActA polypeptides of Listeria ivanovii and Listeria monocytogenes harbor related binding sites for host microfilament proteins
AUTHOR(S): **Gerstel, Birgit**; Groebe, Lothar; Pistor, Susanne; **Chakraborty, Trinad**; Wehland, Juergen
CORPORATE SOURCE: Abteilung Zellbiologie und Immunologie, Gesellschaft Biotechnologische Forschung, Braunschweig, D-38124, Germany
SOURCE: Infect. Immun. (1996), 64(6), 1929-1936
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The surface-bound ActA polypeptide of the intracellular bacterial pathogen Listeria monocytogenes acts as a nucleator protein, generating the actin cytoskeleton around intracellularly motile bacteria. In this work, we examd the functional similarity of ActA from Listeria ivanovii (iActA) ATCC 19119 to its L. monocytogenes counterpart. The amino acid sequence of iActA predicts a mol. mass of 123 kDa and harbors 8 proline-rich repeats. For functional anal., various iActA derivs. and hybrid constructs of L. ivanovii and L. monocytogenes ActA polypeptides were transiently expressed in epithelial cells and examd. for recruitment of host microfilament proteins by a mitochondrial targeting assay. As has been demonstrated with ActA, iActA also spontaneously inserted into the surface of mitochondria and induced recruitment of actin, .alpha.-actinin, and the vasodilator-stimulated phosphoprotein (VASP) to these subcellular organelles. By comparison of amino-terminally truncated iActA derivs. for their ability to recruit cytoskeletal proteins, a region essential for actin filament accumulation was identified between amino acid residues 290 and 325. Such derivs., however, retained their ability to bind VASP. Replacement of the proline-rich repeats in Acta with those of iActA also resulted in VASP recruitment. Hence, despite the limited overall sequence homol. between ActA and iActA, the two mols.

consist of at least two similar domains: a highly pos. charged N-terminal domain that is directly involved in actin filament recruitment and a proline-rich repeat region required for VASP binding.

L36 ANSWER 17 OF 26 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 13
ACCESSION NUMBER: 1996:320196 CAPLUS
DOCUMENT NUMBER: 125:2892
TITLE: An Escherichia coli hemolysin transport system-based vector for the export of polypeptides: export of Shiga-like toxin IIeB subunit by **Salmonella typhimurium** aroA
AUTHOR(S): Tzschaschel, Barbara D.; **Guzman, Carlos A.**; **Timmis, Kenneth N.**; de Lorenzo, Victor
CORPORATE SOURCE: Division Microbiology, GBF-National Research Centre Biotechnology, Braunschweig, 38124, Germany
SOURCE: Nat. Biotechnol. (1996), 14(6), 765-769
CODEN: NABIF9; ISSN: 1087-0156
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The export of Escherichia coli hemolysin across the cytoplasmic and the outer membranes requires the COOH-terminal signal sequence of HlyA, the two specific translocator proteins HlyB and HlyD, and the outer membrane protein TolC. We have developed an export cloning system that is composed of 2 vectors: one in which the fusion of the desired gene with the 3'-end of hlyA is generated, and a second in which the sequences contg. the fusion are combined with the accessory genes hlyB and hlyD, thereby reconstructing the natural organization of the **hly** focus. In the second vector the fusion and the accessory genes are flanked by NotI sites, allowing subcloning of the whole cluster into a variety of minitransposons to achieve the stable integration of the constructs into the chromosome of gram-neg. bacteria. Since some applications may require the prodn. of transcription fusions, an alternative version of the system provides the efficient translation initiation region of T7 phase gene 10 upstream of the fusion protein coding sequence. The usefulness of the system was assessed by constructing a fusion between the gene encoding the B subunit of Shiga-like toxin Ile and the 3'-end of hlyA. An attenuated **Salmonella typhimurium** vaccine strain harboring the resulting construct, either in multicopy or monocopy, efficiently expressed and exported the chimeric protein. We anticipate that this system will lead to a higher stability of the engineered function and permit a faithful monitoring of the export of the recombinant peptide under physiol. single-copy conditions.

L36 ANSWER 18 OF 26 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1996:391586 BIOSIS
DOCUMENT NUMBER: PREV199699113942
TITLE: Generation and characterisation of antibodies against the metalloprotease (MpI) from Listeria monocytogenes.
AUTHOR(S): Broer, J. (1); **Gerstel, B. (1)**; Domann, E.; **Chakraborty, T.**; Wehland, J. (1)
CORPORATE SOURCE: (1) Abt. Zellbiologie Immunbiologie, GBF, D-33124

Braunschweig Germany
SOURCE: European Journal of Cell Biology, (1996) Vol. 69, No. SUPPL. 42, pp. 146.
Meeting Info.: 21st Annual Meeting of the German Society for Cell Biology Hamburg, Germany March 24-28, 1996
ISSN: 0171-9335.
DOCUMENT TYPE: Conference
LANGUAGE: English

L36 ANSWER 19 OF 26 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 14
ACCESSION NUMBER: 1996:252984 CAPLUS
DOCUMENT NUMBER: 124:314008
TITLE: Apoptosis of mouse dendritic cells is triggered by listeriolysin, the major virulence determinant of Listeria monocytogenes
AUTHOR(S): Guzman, Carlos A.; Domann, Eugen; Rohde, Manfred; Bruder, Dunja; Darji, Ayub; **Weiss, Siegfried**; Wehland, Juergen; **Chakraborty, Trinad**; **Timmis, Kenneth N.**
CORPORATE SOURCE: Div. Microbiol., National Res. Centre Biotechnol., Braunschweig, 38124, Germany
SOURCE: Mol. Microbiol. (1996), 20(1), 119-26
CODEN: MOMIEE; ISSN: 0950-382X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Infection of a murine-spleen dendritic cell line by Listeria monocytogenes was found to induce cell death through apoptosis. To characterize the bacterial product(s) involved in induction of apoptosis, dendritic cells were infected with the L. monocytogenes EGD strain and several isogenic mutants deficient in the prodn. of individual listerial virulence factors. The ability to induce cellular apoptosis was retained by all mutants tested, except the prfA and .DELTA.hly mutants, both of which are unable to produce listeriolysin. Apoptosis was also induced by purified listeriolysin suggesting that this protein directly induces apoptosis. Purified recombinant listeriolysins rendered either weakly hemolytic by a C-484 to S mutation, or nonhemolytic by a W-491 to A mutation exhibited little or no capacity to induce apoptosis, indicating that both activities are assocd. within the same protein region. Treatment with purified listeriolysin or L. monocytogenes infection also triggers apoptosis in explanted bone-marrow dendritic cells. Thus invasion of dendritic cells by L. monocytogenes, which results in cell death, may play an important role in the pathogenesis of listerial infections by impairing immune responses, hindering bacterial clearance and promoting spread of the infection.

L36 ANSWER 20 OF 26 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1996:391282 BIOSIS
DOCUMENT NUMBER: PREV199699113638
TITLE: The ActA-polypeptide of L. ivanovii harbours two distinct domains required for the interaction with the host microfilament system.
AUTHOR(S): Groebe, Lothar (1); **Gerstel, Birgit (1)**; Pistor, Susanne (1); **Chakraborty, Trinad**; Wehland, Juergen (1)
CORPORATE SOURCE: (1) Abteilung Zellbiologie Immunologie, GBF, D-38124

Braunschweig Germany
SOURCE: European Journal of Cell Biology, (1996) Vol. 69, No. SUPPL. 42, pp. 45.
Meeting Info.: 21st Annual Meeting of the German Society for Cell Biology Hamburg, Germany March 24-28, 1996
ISSN: 0171-9335.
DOCUMENT TYPE: Conference
LANGUAGE: English

L36 ANSWER 21 OF 26 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 15
ACCESSION NUMBER: 1995:781454 CAPLUS
DOCUMENT NUMBER: 123:196435
TITLE: Interaction of Listeria monocytogenes with mouse dendritic cells
AUTHOR(S): Guzman, Carlos A.; Rohde, Manfred; **Chakraborty, Trinad**; Domann, Eugen; Hudel, Martina; Wehland, Juergen; **Timmis, Kenneth N.**
CORPORATE SOURCE: Div. Microbiol., GBF-National Res. Cent. Biotechnol., Braunschweig, Germany
SOURCE: Infect. Immun. (1995), 63(9), 3665-73
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In this, study, the interaction of murine dendritic cells with Listeria monocytogenes was investigated. Dendritic cells are efficient antigen-presenting cells, play a key role in the immune response, and are capable of migrating over substantial distances between sites of infection and lymphoid tissues. L. monocytogenes EGD invaded dendritic cells, escaped from phagosomes into the cytoplasm, and there directed actin nucleation, polymn., and polarization in a typical fashion, thereby achieving intracellular movement and cell-to-cell spread. The internalization process appears to be independent of the inl locus. Interestingly, an intact microtubular function was essential for efficient uptake, whereas in a previous report, microtubule disruption did not affect bacterial spread in Caco-2 cells. The results obtained also suggest that L. monocytogenes binds to glycosylated receptors of dendritic cells. Uptake of Listeria cells was mediated by a protein kinase-dependent transducing phosphorylation signal that induces the actin polymn.-dependent phagocytic process. To achieve efficient uptake, de novo protein synthesis of eukaryotic and prokaryotic cells is also required. Despite the killing of dendritic cells, wild-type bacteria were found to persist in small nos. in some cells for at least 24 h. When different isogenic mutants of the EGD strains were analyzed for their capability to interact with dendritic cells, it was obsd. that some virulence-attenuated mutants (i.e., prfA and .DELTA.hly) persisted in large nos. for even longer times. Invasion of dendritic cells by L. monocytogenes, which in turn could result in either cell death or persistent infection, might have an important role in the pathogenesis of listeriosis, leading to impaired immune responses with inefficient bacterial clearance and/or promoting bacterial spread.

L36 ANSWER 22 OF 26 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 16
ACCESSION NUMBER: 1995:550195 CAPLUS
DOCUMENT NUMBER: 123:7302

TITLE: A focal adhesion factor directly linking intracellularly motile Listeria monocytogenes and Listeria ivanovii to the actin-based cytoskeleton of mammalian cells

AUTHOR(S): **Chakraborty, Trinad**; Ebel, Frank; Domann, Eugen; Niebuhr, Kirsten; **Gerstel, Birgit**; Pistor, Susanne; Temm-Grove, Constance J.; Jockusch, Brigitte M.; Reinhard, Matthias; et al.

CORPORATE SOURCE: Institut Medizinische Mikrobiologie, Giessen, D-35392, Germany

SOURCE: EMBO J. (1995), 14(7), 1314-21
CODEN: EMJODG; ISSN: 0261-4189

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The surface-bound ActA polypeptide of the intracellular bacterial pathogen Listeria monocytogenes is the sole listerial factor needed for recruitment of host actin filaments by intracellularly motile bacteria. Here, the authors report that following Listeria infection the host vasodilator-stimulated phosphoprotein (VASP), a microfilament- and focal adhesion-assocd. substrate of both the cAMP- and cGMP-dependent protein kinases, accumulates on the surface of intracytoplasmic bacteria prior to the detection of F-actin 'clouds'. VASP remains assocd. with the surface of highly motile bacteria, where it is polarly located, juxtaposed between one extremity of the bacterial surface and the front of the actin comet tail. Since actin filament polymn. occurs only at the very front of the tail, VASP exhibits properties of a host protein required to promote actin polymn. Purified VASP binds directly to the ActA polypeptide in vitro. A ligand-overlay blot using purified radiolabeled VASP enabled the authors to identify the ActA homolog of the related intracellular motile pathogen, Listeria ivanovii, as a protein with a mol. mass of .apprx.150 kDa. VASP also assocs. with actin filaments recruited by another intracellularly motile bacterial pathogen, Shigella flexneri. Hence, by the simple expedient of expressing surface-bound attractor mols., bacterial pathogens effectively harness cytoskeletal components to achieve intracellular movement.

L36 ANSWER 23 OF 26 WPIDS COPYRIGHT 2002 DERWENT INFORMATION LTD

ACCESSION NUMBER: 1993-265726 [34] WPIDS

DOC. NO. CPI: C1993-118430

TITLE: DNA-hybrids of B-sub units of bacterial toxins and haemolysin A - useful as vaccine for shiga guanidine and related toxins.

DERWENT CLASS: B04 D16

INVENTOR(S): BRAHMBHATT, H N; SU, G; **TIMMIS, K N**; WEHLAND, J; BRAHMBHATT, H

PATENT ASSIGNEE(S): (GBFB) GBF GES BIOTECH FORSCHUNG GMBH

COUNTRY COUNT: 20

PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
DE 4219696	A1	19930819	(199334)*		14
WO 9316186	A1	19930819	(199334)		

RW: AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
W: AU JP US

AU 9336282	A	19930903 (199401)	
EP 627006	A1	19941207 (199502)	GE
R: AT BE CH DE DK ES FR GB IT LI NL SE			
JP 07505052	W	19950608 (199531)	
AU 672388	B	19961003 (199708)	
EP 627006	B1	19970502 (199722)	GE 16
R: AT BE CH DE DK ES FR GB IT LI NL SE			
DE 59306342	G	19970605 (199728)	
ES 2104130	T3	19971001 (199746)	

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
DE 4219696	A1	DE 1992-4219696	19920616
WO 9316186	A1	WO 1993-EP348	19930212
AU 9336282	A	AU 1993-36282	19930212
EP 627006	A1	EP 1993-905242	19930212
		WO 1993-EP348	19930212
JP 07505052	W	JP 1993-513792	19930212
		WO 1993-EP348	19930212
AU 672388	B	AU 1993-36282	19930212
EP 627006	B1	EP 1993-905242	19930212
		WO 1993-EP348	19930212
DE 59306342	G	DE 1993-506342	19930212
		EP 1993-905242	19930212
		WO 1993-EP348	19930212
ES 2104130	T3	EP 1993-905242	19930212

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9336282	A	Based on	WO 9316186
EP 627006	A1	Based on	WO 9316186
JP 07505052	W	Based on	WO 9316186
AU 672388	B	Previous Publ.	AU 9336282
		Based on	WO 9316186
EP 627006	B1	Based on	WO 9316186
DE 59306342	G	Based on	EP 627006
		Based on	WO 9316186
ES 2104130	T3	Based on	EP 627006

PRIORITY APPLN. INFO: DE 1992-4204737 19920217; DE 1992-4219696 19920616

AN 1993-265726 [34] WPIDS

AB DE 4219696 A UPAB: 19931123

Claimed is a hybrid DNA comprising a B-subunit of a bacterial toxin fused with DNA encoding haemolysin A (**HLY** A) or its C-terminal fragment. Also claimed is the oligo hybrid peptide comprising the subunit of a bacterial toxin and **HLY** A or its C-terminal fragment.

The toxin is pref. shigatoxin or shiga-like toxin, i.e. SLT-II or SLT-IIv. The DNA pref. has a promoter for expression in Salmonella (**S.**) **typhimurium** (t.), e.g. S. t. aroA-SL3261 (Heisetz and Stocker, 1981, Nature 291, 238-239). Also claimed is a plasmid contg. the hybrid DNA.

USE/ADVANTAGE - The new oligohybridpeptide is used as a vaccine

(calimed) for shiga and related toxins. The hybrid is secreted by S. t. aroA-3261 allowing high expression without being toxic for the host strain. In contrast to prior art systems using fusions with the LamB-protein, **HLY** A fusions allow larger polypeptides to be expressed.
Dwg.0/6

ABEQ WO 9316186 A UPAB: 19931119
Claimed is a hybrid DNA comprising a B-subunit of a bacterial toxin fused with DNA encoding haemolysin A (**HLY** A) or its C-terminal fragment. Also claimed is the oligo hybrid peptide comprising the subunit of a bacterial toxin and **HLY** A or its C-terminal fragment.
The toxin is pref. shigatoxin or shiga-like toxin, i.e. SLT-II or SLT-IIv. The DNA pref. has a promoter for expression in Salmonella (**S.**) **typhimurium** (t.), e.g. S. t. aroA-SL3261 (Heisetz and Stocker, 1981, Nature 291, 238-239). Also claimed is a plasmid contg. the hybrid DNA.
USE/ADVANTAGE - The new oligohybridpeptide is used as a vaccine (calimed) for shiga and related toxins. The hybrid is secreted by S. t. aroA-3261 allowing high expression without being toxic for the host strain. In contrast to prior art systems using fusions with the LamB-protein, **HLY** A fusions allow larger polypeptides to be expressed.

ABEQ EP 627006 B UPAB: 19970530
Hybrid DNA, wherein a DNA structure coding for a fragment of a bacterial toxin is fused with a C-terminal fragment of a DNA structure coding for HlyA (haemolysin), characterized in that the fragment of the bacterial toxin is subunit B of Shigatoxin or Shiga-like toxins.
Dwg.0/6

L36 ANSWER 24 OF 26 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 17
ACCESSION NUMBER: 1985:107849 CAPLUS
DOCUMENT NUMBER: 102:107849
TITLE: Cloning of enterotoxin gene from Aeromonas hydrophila provides conclusive evidence of production of a cytotonic enterotoxin
AUTHOR(S): **Chakraborty, T.**; Montenegro, M. A.; Sanyal, S. C.; Helmuth, R.; Bulling, E.; **Timmis, K. N.**
CORPORATE SOURCE: Bundesgesundheitsamt, West Berlin, Fed. Rep. Ger.
SOURCE: Infect. Immun. (1984), 46(2), 435-41
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Culture filtrates of 2 A. hydrophila strains which were isolated from patients with diarrhea and assumed to be causative agents of the infections were shown to contain enterotoxic, cytotoxic, and hemolytic activities. Modest heat treatment of the filtrates inactivated the cytotoxic and cytolytic activities, but not the enterotoxic activity. The construction of cosmid gene banks in Escherichia coli of DNA from both A. hydrophila strains demonstrated that the determinants of the 3 activities are located on 3 different segments of the A. hydrophila chromosome. Both heated culture filtrates of A. hydrophila and nonheated filtrates of an E. coli clone contg. the A. hydrophila enterotoxin gene provoked fluid accumulation in the rabbit ileal loop and suckling mouse models and

caused elongation of Chinese hamster ovary cells. Differences in the responses of the models to the A. hydrophila enterotoxin and to the heat-labile and heat-stable toxins of E. coli indicated that the former is distinct from the latter 2 types of toxin. These results constitute conclusive evidence for the prodn. by A. hydrophila of a cytotonic enterotoxin that is distinct from the A. hydrophila cytotoxin and hemolysin and known E. coli enterotoxins.

L36 ANSWER 25 OF 26 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 18
ACCESSION NUMBER: 1984:584957 CAPLUS
DOCUMENT NUMBER: 101:184957
TITLE: Genetics of toxin synthesis in pathogenic gram-negative enteric bacteria
AUTHOR(S): **Timmis, K. N.**; Montenegro, M. A.; Bulling, E.; **Chakraborty, T.**; Sanyal, S.
CORPORATE SOURCE: Dep. Med. Biochem., Univ. Geneva, Geneva, Switz.
SOURCE: FEMS Symp. (1984), 24(Bact. Protein Toxins), 13-27
CODEN: FEMSDW; ISSN: 0163-9188
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with 48 refs.

L36 ANSWER 26 OF 26 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC. DUPLICATE 19
ACCESSION NUMBER: 1983:52961 BIOSIS
DOCUMENT NUMBER: BR24:52961
TITLE: NO MORE ANIMAL EXPERIMENTS IN DEMONSTRATION OF ENTERO TOXINS.
AUTHOR(S): **CHAKRABORTY T**; HELMUTH R; **TIMMIS K**; SANYAL S C; BULLING E
CORPORATE SOURCE: FED. HEALTH OFFICE, INST. VET. MED., BERLIN, FRG.
SOURCE: INTERNATIONAL UNION OF MICROBIOLOGICAL SOCIETIES. 13TH INTERNATIONAL CONGRESS OF MICROBIOLOGY; BOSTON, MASS., USA, AUG. 8-13, 1982. XIV+182P. AMERICAN SOCIETY FOR MICROBIOLOGY: WASHINGTON, D.C., USA. PAPER, (1982) 0 (0), P110.
ISBN: 0-914826-44-1.
DOCUMENT TYPE: Conference
FILE SEGMENT: BR; OLD
LANGUAGE: English

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